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(21) Applicant: TAKARA SHUZO CO. LTD.  
609 Takenaka-cho  
Fushimi-ku,  
Kyoto-shi,  
Kyoto-fu (JP)

(21) Inventor: Takashi, Okado  
4-36-312, Honmaru-cho  
Otsu-shi,  
Shiga-ken (JP)  
Inventor: Kazutoh, Takesako  
4-20-208, Akibadai  
Otsu-shi,  
Shiga-ken (JP)  
Inventor: Ikunoshin, Kato  
1-1-150, Nanryo-cho  
Uji-Shi,  
Kyoto-fu (JP)

(74) Representative: Wakerley, Helen Rachael  
Reddie & Grose,  
16 Theobalds Road  
London WC1X 8PL (GB)

(52) Gene coding for a protein regulating aureobasidin sensitivity.

(57) An isolated gene coding for a protein regulating aureobasidin sensitivity. A process for cloning the gene with the use of the gene or a part of the same as a probe. A nucleic acid probe being hybridizable with the gene. An antisense DNA or RNA of the gene. A recombinant or transformant having the gene contained therein. An isolated protein regulating aureobasidin sensitivity and a process for producing the same by using the transformant. An antibody for the protein. A process for detecting the protein or the gene. A process for screening an antimycotic by using the protein or the transformant. This invention is useful in the diagnosis and treatment of diseases including mycoses.

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This invention relates to a protein regulating the sensitivity to an antimycotic aureobasidin and a gene coding for this protein, namely, a gene coding for a protein regulating aureobasidin sensitivity. The present invention further relates to a series of the uses of the protein and the gene. Furthermore, it relates to an antibody against this protein and the use of the same.

5 Systemic mycoses including candidiasis have increased with an increase in immunocompromised patients in recent years due to, for example, the extended use of immunosuppressive drugs and acquired immunodeficiency syndrome (AIDS), and as opportunistic infection due to microbial substitution caused by the frequent use of wide-spectrum antibacterial antibiotics. Although drugs for treating mycoses such as amphotericin B, flucytosine and azole drugs (for example, fluconazole and miconazole) are now used to 10 cope with this situation, none of them can achieve a satisfactory effect. Also, known diagnostic drugs are insufficient. For candidiasis, in particular, although there have been known several diagnostic drugs (for example, CAND-TEC for detection of candida antigen and LABOFIT for detection of D-arabinitol), none of them gives any satisfactory results in specificity or sensitivity.

15 The reasons for the delay in the development of remedies and diagnostic drugs for mycoses as described above are that fungi causing the mycoses are eukaryotic organisms similar to the host (i.e., man) and thus not largely different from man and that knowledges of fungi, in particular, pathogenic fungi are insufficient. Therefore it is difficult to distinguish fungi from man or to selectively kill fungi, which is responsible for the delay in the development of drugs for mycoses.

20 Recently the application of genetic engineering techniques such as antisense or PCR to the treatment and diagnosis of mycoses has been expected. However known genes which are applicable thereto and or proteins coded for by these genes are rare (PCT Pamphlet W092 03455). Regarding pathogenic fungi, there have been cloned in recent years an acid protease gene, which has been assumed to participate in the pathogenicity of Candida albicans (hereinafter referred to simply as C. albicans) and Candida tropicalis - (hereinafter referred to as C. tropicalis) causing candidiasis [B. Hube et al., J. Med. Vet. Mycol., 29, 129 - 25 132 (1991); Japanese Patent Laid-Open No. 49476 1993; and G. Togni et al., FEBS Letters, 286, 181 - 185 (1991)], a calmodulin gene of C. albicans [S.M. Saporito et al., Gene, 106, 43 - 49 (1991)] and a glycolytic pathway enzyme enolase gene of C. albicans [P. Sundstrom et al., J. Bacteriology, 174, 6789 - 6799 (1991)]. However, each of these genes and proteins coded for thereby is either indistinguishable from nonpathogenic fungi and eukaryotic organisms other than fungi or, if distinguishable therefrom, cannot serve 30 as a definite action point for exhibiting any selective toxicity. Aureobasidin [Japanese Patent Laid-Open No. 138296 1990, No. 22995 1991, No. 220199 1991 and No. 279384 1993, Japanese Patent Application No. 303177 1992, J. Antibiotics, 44 (9), 919 - 924, ibid., 44 (9), 925 - 933, ibid., 44 (11), 1187 - 1198 (1991)] is a cyclic depsipeptide obtained as a fermentation product of a strain Aureobasidium pullulans No. R106. It is completely different in structure from other antimycotics. As Tables 1 and 2 show, aureobasidin A, which is 35 a typical aureobasidin compound, exerts a potent antimycotic activity on various yeasts of the genus Candida including C. albicans which is a pathogenic fungus, Cryptococcus neoformans, Histoplasma capsulatum, Blastomyces dermatitidis and fungi of the genus Aspergillus (Japanese Patent Laid-open No. 138296 1990) but has an extremely low toxicity in mammal. Thus this compound is expected to be useful as an antimycotic being excellent in selective toxicity.

40 Hereinafter, Candida, Cryptococcus and Aspergillus will be abbreviated respectively as C., Cr. and A.

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Table 1

Test strain	TIMM No.	MIC( $\mu$ g/ml)
<u>C. albicans</u>	0136	$\leq 0.04$
<u>C. albicans</u> var. <u>stellatoidea</u>	1308	$\leq 0.04$
<u>C. tropicalis</u>	0312	0.08
<u>C. kefyr</u>	0298	0.16
<u>C. parapsilosis</u>	0287	0.16
<u>C. krusei</u>	0270	$\leq 0.04$
<u>C. guilliermondii</u>	0257	0.08
<u>C. glabrata</u>	1062	$\leq 0.04$
<u>Cr. neoformans</u>	0354	0.63
<u>Cr. terreus</u>	0424	0.31
<u>Rhodotorula rubra</u>	0923	0.63
<u>A. fumigatus</u>	0063	20
<u>A. clavatus</u>	0056	0.16

Table 2

Test strain	TIMM No.	MIC( $\mu$ g/ml)
<u>A. nidulans</u>	0112	0.16
<u>A. terreus</u>	0120	5
<u>Penicillium commune</u>	1331	1.25
<u>Trichophyton mentagrophytes</u>	1189	10
<u>Epidermophyton floccosum</u>	0431	2.5
<u>Fonsecaea pedrosoi</u>	0482	0.31
<u>Exophiala werneckii</u>	1334	1.25
<u>Cladosporium bantianum</u>	0343	0.63
<u>Histoplasma capsulatum</u>	0713	0.16
<u>Paracoccidioides brasiliensis</u>	0880	0.31
<u>Geotrichum candidum</u>	0694	0.63
<u>Blastomyces dermatitidis</u>	0126	0.31

## [Problems to be Solved by the Invention]

Each of the conventional antimycotics with a weak toxicity shows only a fungistatic effect, which has been regarded as a clinical problem. In contrast, aureobasidin has a fungicidal effect. From this point of view, it has been urgently required to clarify the mechanism of the selective toxicity to fungi of aureobasidin. However this mechanism still remains unknown.

Under these circumstances, the present invention aims at finding a novel protein regulating aureobasidin sensitivity through the clarification of the mechanism of the selective toxicity to fungi of aureobasidin. Accordingly, the present invention aims at finding a gene coding for a protein regulating aureobasidin sensitivity, providing a process for cloning this gene and the protein regulating aureobasidin sensitivity which is encoded by this gene, further providing an antisense DNA and an antisense RNA of this gene, providing a nucleic acid probe being hybridizable with this gene, providing a process for detecting this gene with the use of the nucleic acid probe, providing a process for producing the protein regulating aureobasidin sensitivity by using this gene and providing an antibody against the protein regulating aureobasidin sensitivity, and a process for detecting the protein regulating aureobasidin sensitivity by using this antibody.

## [Means for Solving the Problems]

The present invention may be summarized as follows. Namely, the first aspect of the present invention relates to an isolated gene coding for a protein regulating aureobasidin sensitivity, that is, a gene regulating aureobasidin sensitivity. The second aspect of the invention relates to a process for cloning a gene regulating aureobasidin sensitivity which is characterized by using the gene regulating aureobasidin sensitivity of the first aspect or a part thereof as a probe. The third aspect of the invention relates to a nucleic acid probe which is hybridizable with a gene regulating aureobasidin sensitivity and comprises a sequence consisting of 15 or more bases. The fourth aspect of the invention relates to an antisense DNA of a gene regulating aureobasidin sensitivity. The fifth aspect of the invention relates to an antisense RNA of a gene regulating aureobasidin sensitivity. The sixth aspect of the invention relates to a recombinant plasmid having a gene regulating aureobasidin sensitivity contained therein. The seventh aspect of the invention relates to transformant having the above-mentioned plasmid introduced thereto. The eighth aspect of the invention relates to a process for producing a protein regulating aureobasidin sensitivity by using the above-mentioned transformant. The ninth aspect of the invention relates to an isolated protein regulating aureobasidin sensitivity. The tenth aspect of the invention relates to an antibody against a protein regulating aureobasidin sensitivity. The eleventh aspect of the invention relates to a process for detecting a protein regulating aureobasidin sensitivity by using the above-mentioned antibody. The twelfth aspect of the invention relates to a process for detecting a gene regulating aureobasidin sensitivity by the hybridization which is characterized by using the nucleic acid probe of the third aspect of the present invention. The thirteenth aspect of the invention relates to a process for screening an antimycotic by using the above-mentioned transformant or a protein regulating aureobasidin sensitivity.

The present inventors have found out that fungi such as Schizosaccharomyces pombe (hereinafter referred to simply as Schizo. pombe) and Saccharomyces cerevisiae (hereinafter referred to simply as S. cerevisiae) and, further, mammalian cells such as mouse lymphoma EL-4 cells are sensitive to aureobasidin, as Table 3 shows.

Table 3

30	Test strain or cell	MIC(μg/ml)
	<u>Schizo. pombe</u>	0.08
	<u>S. cerevisiae</u>	0.31
35	mouse lymphoma EL-4	10
	mouse lymphoma L5178Y	100
	NRK-49F	12.5

The present inventors have mutagenized a wild-type strain of Schizo. pombe or S. cerevisiae, sensitive to aureobasidin, to thereby give resistant mutants. We have further successfully isolated a gene capable of conferring aureobasidin resistance (a resistant gene) from these resistant mutants and another gene capable of imparting aureobasidin sensitivity (a sensitive gene) from the corresponding sensitive cells. Furthermore, We have disclosed the existence of a protein encoded by each of these genes. By culturing cells which have been transformed by introducing the above-mentioned gene, We have succeeded in the expression of this gene. Furthermore, We have successfully found out a novel gene regulating aureobasidin sensitivity from another fungus being sensitive to aureobasidin by using a DNA fragment of the above-mentioned gene as a probe. In addition, We have clarified that the gene regulating aureobasidin sensitivity is essentially required for the growth of the cells and found out that the detection of this gene or a protein which is a gene product thereof with an antibody enables the diagnosis of diseases caused by these cells, for example, mycoses induced by fungi, and that an antisense DNA or an antisense RNA, which inhibits the expression of the gene regulating aureobasidin sensitivity being characteristic to the cells, is usable as a remedy for diseases caused by these cells, for example, mycoses induced by fungi, thus completing the present invention.

That is to say, pathogenic fungi listed in Tables 1 and 2 and fungi and mammalian cells listed in Table 3, each having a sensitivity to aureobasidin, each carries a protein regulating aureobasidin sensitivity and a gene coding for this protein. The term "a protein regulating aureobasidin sensitivity" as used herein means a protein which is contained in an organism having a sensitivity to aureobasidin. This protein is required for the expression of the sensitivity or resistance to aureobasidin. As a matter of course, a protein having 35% or more homology with the above-mentioned protein and having a similar function is also a member of the

protein regulating aureobasidin sensitivity according to the present invention. Furthermore, proteins obtained by modifying these proteins by the genetic engineering procedure are members of the protein regulating aureobasidin sensitivity according to the present invention. A gene regulating aureobasidin sensitivity means a gene which codes for such a protein regulating aureobasidin sensitivity as those described above and 5 involves both of sensitive genes and resistant genes.

The first aspect of the present invention relates to a gene regulating aureobasidin sensitivity. This gene can be isolated in the following manner. First, aureobasidin sensitive cells (a wild-type strain) is mutagenized to thereby induce a resistant strain. From chromosome DNA or cDNA of this resistant strain, a DNA library is prepared and a gene capable of conferring a resistance (a resistant gene) is cloned from this 10 library. Then a DNA library of a wild strain is prepared and a DNA molecule being hybridizable with the resistant gene is isolated from this library and cloned. Thus a sensitive gene can be isolated.

The mutagenesis is performed by, for example, treating with a chemical such as ethylmethane sulfonate (EMS) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or by ultraviolet or other radiation. The cell that has 15 acquired the resistance can be screened by culturing the mutagenized cells in a nutritional medium containing aureobasidin at an appropriate concentration under appropriate conditions. The resistant strain thus obtained may vary depending on the method and conditions selected for the mutagenesis. Also, strains differing in the extent of resistance from each other can be separated by changing the aureobasidin concentration or a temperature-sensitive resistant strain can be isolated by changing the temperature in the 20 step of screening. There are a number of mechanisms of resistance to aureobasidin. Accordingly, a number of resistant genes can be isolated by genetically classifying these various resistant strains. In the case of a yeast, the classification may be performed by the complementation test. Namely, resistant strains are prepared from haploid cells. Next, diploid cells can be obtained by crossing resistant strains differing in mating type from each other. Then spores formed from these diploids are examined by the tetrad analysis.

As typical examples of the genes regulating aureobasidin sensitivity (named aur) according to the 25 present invention, aur1 and aur2 genes may be cited. Typical examples of the aur1 gene include spaur1 gene isolated from Schizo. pombe and scaur1 gene isolated from S. cerevisiae, while typical examples of the aur2 gene include scaur2 gene isolated from S. cerevisiae. Now, resistant genes (spaur1<sup>R</sup>, scaur1<sup>R</sup> and scaur2<sup>R</sup>) isolated from resistant mutants by the present inventors and sensitive genes (spaur1<sup>S</sup>, scaur1<sup>S</sup> and scaur2<sup>S</sup>) isolated from sensitive wild-type strains will be described.

30 Fig. 1 shows a restriction enzyme map of the genes spaur1<sup>R</sup> and spaur1<sup>S</sup> regulating aureobasidin sensitivity, Fig. 2 shows a restriction enzyme map of scaur1<sup>R</sup> and scaur1<sup>S</sup> and Fig. 3 shows a restriction enzyme map of scaur2<sup>R</sup> and scaur2<sup>S</sup>.

Schizo. pombe, which is sensitive to aureobasidin, is mutagenized with EMS and a genomic library of the resistant stain thus obtained is prepared. From this library, a DNA fragment containing a resistant gene 35 (spaur1<sup>R</sup>) and having the restriction enzyme map of Fig. 1 is isolated. This gene has a nucleotide sequence represented by SEQ ID No. 1 in Sequence Listing. The amino acid sequence of a protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 2 in Sequence Listing. By the hybridization with the use of this resistant gene as a probe, a DNA fragment containing a sensitive gene (spaur1<sup>S</sup>) and having the restriction enzyme map of Fig. 1 is isolated from a 40 sensitive strain. This gene has a nucleotide sequence represented by SEQ ID No. 3 in Sequence Listing. The amino acid sequence of a protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 4 in Sequence Listing. A comparison between the sequences of SEQ ID No. 3 and SEQ ID No. 1 reveals that a mutation from G to T occurs at the base at the position 1053, while a comparison between the sequences of SEQ ID No. 4 and SEQ ID No. 2 reveals 45 that glycine at the residue 240 is converted into cysteine at the amino acid level, thus giving rise to the resistance.

Also, S. cerevisiae, which is sensitive to aureobasidin, is mutagenized with EMS and genomic libraries of two resistant strains thus obtained are prepared. From one of these libraries, a DNA fragment containing a resistant gene (scaur1<sup>R</sup>) as a dominant mutant and having the restriction enzyme map of Fig. 2 is 50 isolated, while a DNA fragment containing a resistant gene (scaur2<sup>R</sup>) and having the restriction enzyme map of Fig. 3 is isolated from another library.

The nucleotide sequence of the coding region for the protein of the scaur1<sup>R</sup> gene is the one represented by SEQ ID No. 5 in Sequence Listing. The amino acid sequence of the protein encoded by this gene, which is estimated on the basis of the above nucleotide sequence, is the one represented by SEQ ID 55 No. 6 in Sequence Listing. By the hybridization with the use of this resistant gene scaur1<sup>R</sup> as a probe, a DNA fragment containing a sensitive gene (scaur1<sup>S</sup>) and having the restriction enzyme map of Fig. 2 is isolated from a sensitive strain. This gene has a nucleotide sequence represented by SEQ ID No. 7 in Sequence Listing. The amino acid sequence of a protein encoded by this gene, which is estimated on the

basis of this nucleotide sequence, is the one represented by SEQ ID No. 8 in Sequence Listing. A comparison between the sequences of SEQ ID No. 7 and SEQ ID No. 5 reveals that a mutation from T to A occurs at the base at the position 852, while a comparison between the sequences of SEQ ID No. 8 and SEQ ID No. 6 reveals that phenylalanine at the residue 158 is converted into tyrosine at the amino acid 5 level, thus giving rise to the resistance. The spaur1 gene has a 58% homology with the scaur1 gene at the amino acid level. Thus it is obvious that they are genes coding for proteins having similar functions to each other. When genes and proteins being homologous in sequence with the spaur1 and scaur1 genes and with the proteins encoded thereby are searched from a data base, none having a homology of 35% or above is detected. Accordingly, it is clear that these genes and the proteins encoded thereby are novel molecules 10 which have never been known hitherto.

By the hybridization with the use of the DNA fragment of the resistant gene scaur2<sup>R</sup> as a probe, a DNA fragment containing a sensitive gene (scaur2<sup>S</sup>) and having the restriction enzyme map of Fig. 3 is isolated from a sensitive strain.

The nucleotide sequence of this sensitive gene is the one represented by SEQ ID No. 9 in Sequence 15 Listing and the amino acid sequence of the protein encoded by this gene, which is estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 10 in Sequence Listing. As the result of the homology search with the scaur2<sup>S</sup> gene and the protein encoded thereby, it has been found out that cystic fibrosis transmembrane conductance regulator (CFTR) of mammals alone has a homology as low as 20 31%. Compared with this CFTR, however, the part having a high homology is limited to the region around the domain of the nucleotide binding. It is therefore obvious that the protein encoded by the scaur2<sup>S</sup> gene is a protein which is completely different from CFTR in function and has never been known hitherto.

In order to prove the importance of the aur1 gene in the growth of cells, genes for disrupting the aur1 as shown in Fig. 4 and Fig. 5, in which genes coding for orotidine-5'-phosphate decarboxylase (ura4<sup>+</sup> in the case of Schizo. pombe, while URA3 in the case of S. cerevisiae) have been introduced midway in the aur1 25 gene, are prepared. When these aur1 disrupted genes are introduced into Schizo. pombe and S. cerevisiae respectively, the cells having the aur1 disrupted genes cannot grow at all. Thus it has been revealed that these genes and the proteins encoded thereby are essentially required for the growth of the yeast cells.

As the above examples clearly show, a gene regulating aureobasidin sensitivity can be isolated by 30 using a organism having sensitivity to aureobasidin as a starting material and by carrying out the cloning with the use of various mutagenesis methods and or screening methods depending on the organisms or the methods. Also, genes being hybridizable with the above-mentioned genes are involved in the scope of the 35 first aspect of the present invention. A gene regulating aureobasidin sensitivity can be isolated by the following method. The genomic DNA library of an organism having sensitivity to aureobasidin is integrated into, for example, a high-expression vector of a yeast and transformed into the yeast. Then a clone having aureobasidin resistance is selected from the transformants and DNA is recovered from this clone. Thus the 40 resistant gene can be obtained. As a matter of course, genes obtained by modifying some part of the gene regulating aureobasidin sensitivity thus obtained by some chemical or physical methods are involved in the scope of the first aspect of the present invention.

The second aspect of the present invention relates to a process for cloning a gene regulating 45 aureobasidin sensitivity which is characterized by using the gene regulating aureobasidin sensitivity of the first aspect of the present invention or a part thereof as a probe. Namely, by screening by the hybridization method or the polymerase chain reaction (PCR) method with the use of a part (consisting of at least 15 oligonucleotides) or the whole of the gene as obtained above, a gene coding for a protein having a similar function can be isolated.

For example, a pair of primers of SEQ ID No. 11 and SEQ ID No. 12 in Sequence Listing are 50 synthesized on the basis of the DNA nucleotide sequence of the spaur1<sup>R</sup> gene represented by SEQ ID No. 1. Then PCR is performed by using cDNA of C. albicans, which is a pathogenic fungus, as a template with the use of the above-mentioned primers. The PCR is carried out and the PCR products are electrophoresed on an agarose gel and stained with ethidium bromide. In Fig. 6, the lanes 1, 2 and 3 show the results obtained by using cDNA of C. albicans, cDNA of S. cerevisiae and cDNA of Schizo. pombe as a template, respectively. As shown in Fig. 6, a certain DNA fragment is specifically amplified.

By screening the genomic DNA library of C. albicans with the use of this DNA fragment as a probe, a 55 DNA molecule having a gene (caaur1), which has the same function as that of the spaur1 and scaur1 genes and having the restriction enzyme map of Fig. 7 is obtained. The nucleotide sequence of this caaur1 gene is the one represented by SEQ ID No. 13 in Sequence Listing and the amino acid sequence of the protein encoded by this gene, which has been estimated on the basis of the above nucleotide sequence, is the one represented by SEQ ID No. 14 in Sequence Listing. It has a high homology with the proteins encoded by the spaur1 and scaur1 genes.

By screening the genomic DNA library of C. albicans with the use of a DNA fragment comprising the whole length or a part of the scaur2<sup>S</sup> gene represented by SEQ ID No. 9 in Sequence Listing as a probe, a DNA fragment containing gene (caaur2), which has the same function as that of the scaur2 gene, and having the restriction enzyme map of Fig. 8 is obtained. The nucleotide sequence of a part of this caaur2 gene is the one represented by SEQ ID No. 15 in Sequence Listing and the amino acid sequence of the region encoded by this gene, which has been estimated on the basis of this nucleotide sequence, is the one represented by SEQ ID No. 16 in Sequence Listing. It has a high homology with the corresponding region of the protein encoded by the scaur2 gene.

The third aspect of the present invention relates to an oligonucleotide comprising 15 or more bases which serves as the above-mentioned nucleic acid probe and is hybridizable with the gene regulating aureobasidin sensitivity, for example, the DNA fragment having the restriction enzyme map as shown in Fig. 1, Fig. 2 or Fig. 3. This nucleic acid probe is usable in, for example, the hybridization *in situ*, the identification of a tissue wherein the above-mentioned gene can be expressed, and the confirmation of the presence of a gene or mRNA in various vital tissues. This nucleic acid probe can be prepared by ligating the above-mentioned gene or a gene fragment to an appropriate vector, introducing it into a bacterium, allowing it to replicate in the bacterium, extracting from a disrupted cell suspension, cleaving with a restriction enzyme capable of recognizing the vector-ligating site, electrophoresing and then excising from the gel. Alternatively, this nucleic acid probe can be constructed by the chemical synthesis with the use of a DNA synthesizer or gene amplification techniques by PCR on the basis of the nucleotide sequence of SEQ ID. Nos. 1, 3, 5, 7, 9, 13, 15 or 21 in Sequence Listing. This nucleic acid probe can be labeled with a radioisotope or a fluorescent substance to thereby elevate the detection sensitivity at the use.

The fourth aspect of the present invention relates to an antisense DNA of the above-mentioned gene regulating aureobasidin sensitivity, while the fifth aspect of the present invention relates to an antisense RNA thereof. The introduction of the antisense DNA or antisense RNA into cells makes it possible to control the expression of the gene regulating aureobasidin sensitivity.

As examples of the antisense DNA to be introduced, antisense DNAs corresponding to the genes regulating aureobasidin sensitivity of SEQ ID Nos. 1, 3, 5, 7, 9, 13, 15 or 21 in Sequence Listing and some parts thereof may be cited. SEQ ID No. 17 in Sequence Listing shows an example of this antisense DNA. It represents the sequence of an antisense DNA of the gene regulating aureobasidin sensitivity of SEQ ID No. 1 in Sequence Listing. A fragment obtained by appropriately cleaving some part of such an antisense DNA, and a DNA synthesized depending on such an antisense DNA sequence may be used as the antisense DNA.

As examples of the antisense RNA to be introduced, antisense RNAs corresponding to the genes regulating aureobasidin sensitivity of SEQ ID Nos. 1, 3, 5, 7, 9, 13, 15 or 21 in Sequence Listing and some parts thereof may be cited. SEQ ID No. 18 in Sequence Listing shows an example of this antisense RNA. It represents the sequence of an antisense RNA of the gene regulating aureobasidin sensitivity of SEQ ID No. 1 in Sequence Listing. A fragment obtained by appropriately cleaving some part of such an antisense RNA, an RNA synthesized depending on such an antisense RNA sequence, and an RNA prepared with RNA polymerase in an in vitro transcription system by using the DNA corresponding to the gene regulating aureobasidin sensitivity of SEQ ID No. 1 or SEQ ID No. 3 in Sequence Listing or a part thereof may be used as the antisense RNA.

These antisense DNA and antisense RNA may be chemically modified so as to prevent degradation in vivo or to facilitate passage through a cell membrane. A substance capable of inactivating mRNA, for example, ribozyme may be linked thereto. The antisense DNA and antisense RNA thus prepared are usable in the treatment of various diseases such as mycoses accompanied by an increase in the amount of mRNA coding for a protein regulating aureobasidin sensitivity.

The sixth aspect of the present invention relates to a recombinant plasmid having a gene coding for a protein regulating aureobasidin sensitivity being integrated into an appropriate vector. For example, a plasmid, in which a gene regulating aureobasidin sensitivity gene has been integrated into an appropriate yeast vector, is highly useful as a selection marker gene, since a transformant can be easily selected thereby with the guidance of the chemical resistance by using aureobasidin.

Also, the recombinant plasmid can be stably carried by, for example, Escherichia coli. Examples of vectors which are usable in this case include pUC118, pWH5, pAU-PS, TraplexII9 and pTV118. pAU-PS having the scaur1<sup>S</sup> gene integrated therein is named pSPAR1. pWH5 having the scaur1<sup>S</sup> gene integrated therein is named pSCAR1. pWH5 having the scaur2<sup>R</sup> gene integrated therein is named pSCAR1. TraplexII9 vector having the caaur1 gene integrated therein is named pCAAR1. pTV118 vector having a part of the caaur2 gene integrated therein is named pCAAR2N. Each of these recombinant plasmids is transformed into E. coli. It is also possible to express these plasmids in an appropriate host. Such a gene is reduced

exclusively into the open reading frame (ORF) to be translated into a protein by cleaving with an appropriate restriction enzyme, if necessary, and then bound to an appropriate vector. Thus an expression recombinant plasmid can be obtained. When E. coli is used as the host, plasmids such as pTV118 may be used as a vector for the expression plasmid. When a yeast is used as the host, plasmids such as pYES2 may be used as the vector. When mammalian cells are used as the host, plasmids such as pMAMneo may be used as the vector.

5 The seventh aspect of the present invention relates to a transformant having the above-mentioned recombinant plasmid which has been introduced into an appropriate host. As the host, E. coli, yeasts and mammalian cells are usable. E. coli JM109 transformed by pSPAR1 having the spa<sup>1S</sup> gene integrated therein has been named and designated as Escherichia coli JM109 pSPAR1 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken 305, JAPAN), in accordance with the Budapest Treaty under the accession number FERM BP-4485. E. coli HB101 transformed by pSCAR1 having the sca<sup>1S</sup> gene integrated therein has been named and designated as Escherichia coli HB101 pSCAR1 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4483. E. coli HB101 transformed by pSCAR2 having the sca<sup>2R</sup> gene integrated therein has been named and designated as Escherichia coli HB101 pSCAR2 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4484. E. coli HB101 transformed by pCCAR1 having the caa<sup>1S</sup> gene integrated therein has been named and designated as Escherichia coli HB101 pCAAR1 and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4482. E. coli HB101 transformed by pCAAR2N having a part of the caa<sup>2</sup> gene integrated therein has been named and designated as Escherichia coli HB101 pCAAR2N and deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4481.

30 A transformant capable of expressing a protein regulating aureobasidin sensitivity can be obtained by transforming a expression recombinant plasmid into an appropriate host, as described above. For example, a yeast having a recombinant plasmid as shown in Fig. 9 introduced thereinto is usable for this purpose.

35 The eighth aspect of the present invention relates to a process for producing a protein regulating aureobasidin sensitivity which comprises incubating a transformant according to the sixth aspect of the present invention, which contains a gene coding for this protein, in an appropriate nutritional medium, allowing the expression of the protein, then recovering the protein from the cells or the medium and purifying the same. For the expression of the gene coding for this protein, E. coli, a yeast or mammalian cells are employed as a host. When the yeast having the recombinant plasmid of Fig. 9 is incubated in a medium containing galactose, for example, the protein regulating aureobasidin sensitivity which is encoded by the sca<sup>1S</sup> gene can be expressed.

40 The ninth aspect of the present invention relates to an isolated protein regulating aureobasidin sensitivity. As examples of such a protein, those encoded by the above-mentioned spa<sup>1</sup>, sca<sup>1</sup>, sca<sup>2</sup>, caa<sup>1</sup> and caa<sup>2</sup> genes can be cited.

45 The spa<sup>1S</sup> gene codes for a protein having an amino acid sequence represented by SEQ ID No. 4 in Sequence Listing, while the sca<sup>1S</sup> gene codes for a protein having an amino acid sequence represented by SEQ ID No. 8 in Sequence Listing. By the northern hybridization with the use of a DNA fragment of the spa<sup>1</sup> gene as a probe, mRNAs are detected from a sensitive strain (Fig. 10). Thus the expression of the spa<sup>1</sup> gene is confirmed.

50 Fig. 10 is an autoradiogram showing the results of the northern hybridization wherein mRNAs obtained from cells of a sensitive strain of Schizo. pombe in the logarithmic growth phase (lane 1), cells of a resistant strain in the logarithmic growth phase (lane 2), cells of the sensitive strain in the stationary phase (lane 3) and cells of the resistant strain in the stationary phase (lane 4) are electrophoresed on a 1.2% agarose gel containing formaldehyde.

55 The tenth aspect of the present invention relates to an antibody against the above-mentioned protein regulating aureobasidin sensitivity. For example proteins having amino acid sequences of SEQ ID Nos. 2, 4, 6, 8, 10, 14, 16 or 22 in Sequence Listing and peptides comprising some parts of these amino acid sequences may be used as an antigen. The former antigens can be prepared through the expression in a transformant followed by purification, while the latter antigens can be synthesized on, for example, a marketed synthesizer. The antibody is produced by the conventional method. For example, an animal such as a rabbit is immunized with the above-mentioned protein or a peptide fragment together with an adjuvant

to thereby give a polyclonal antibody. A monoclonal antibody can be produced by fusing antibody-producing B cells, which have been obtained by immunizing with an antigen, with myeloma cells, screening hybridomas producing the target antibody, and incubating these cells. As will be described hereinafter, these antibodies are usable in the treatment and diagnosis for animal and human diseases in which the 5 above-mentioned proteins participate, such as mycoses.

For example, a peptide corresponding to the part of the 103- to 113-positions in the amino acid sequence of SEQ ID No. 8 is synthesized on a synthesizer and then bound to a carrier protein. Then a rabbit is immunized therewith and thus a polyclonal antibody is obtained. In the present invention, keyhole limpet hemocyanin (KLH) is used as the carrier protein. Alternatively, bovine serum albumin and ovalbumin 10 are usable therefor.

The eleventh aspect of the present invention relates to a process for detecting a protein regulating aureobasidin sensitivity by using the above-mentioned antibody. The detection can be carried out by detecting the binding of the antibody to the protein or measuring the amount of binding. For example, the protein or the cells producing the same can be detected by treating with a fluorescence-labeled antibody 15 and then observing under a fluorescence microscope. The amount of the antibody bound to the protein can be measured by various known methods. For example, *S. cerevisiae* cells are stained by the immunofluorescent antibody technique by using the above-mentioned antibody and a secondary antibody such as FITC-labeled antirabbit antibody. Thus it is clarified that the protein encoded by the scaur1 gene is distributed all over the cells. Further, a yeast having the recombinant plasmid of Fig. 9 introduced thereinto 20 is incubated in a medium containing glucose or galactose. The cells thus obtained are disrupted with glass beads and proteins are solubilized. Then these proteins are separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the western blotting is carried out in the conventional manner by using the above-mentioned polyclonal antibody and peroxidase-labeled anti-rabbit antibody. Consequently, the protein encoded by the scaur1 gene can be detected, as Fig. 11 shows.

25 Fig. 11 shows the results of the western blotting wherein the proteins prepared from the cells obtained by the incubation in the presence of glucose (lane 1) or galactose (lane 2) are subjected to SDS-PAGE. A main band binding to the polyclonal antibody of the present invention is detected at around 38 kDa.

The twelfth aspect of the present invention relates to a process for detecting a gene regulating aureobasidin sensitivity, for example, mRNA at the expression of a protein, by using the above-mentioned 30 oligonucleotide as a nucleic acid probe. This process is applicable to the diagnosis for various diseases, including mycoses, associated with an abnormal amount of mRNA coding for the protein. For example, nucleic acids are precipitated from disrupted cells and mRNA is hybridized with a radioisotope-labeled 35 nucleic acid probe on a nitrocellulose membrane. The amount of binding can be measured by autoradiography (Fig. 10) or with a scintillation counter.

The thirteenth aspect of the present invention relates to a process for efficient screening of a novel 40 antimycotic by using the transformant of the seventh aspect of the present invention or the protein regulating aureobasidin sensitivity of the ninth aspect of the present invention. For example, a drug exerting its effect on the protein or the gene of the present invention can be efficiently found out through a comparison of the activity on a transformant containing a sensitive gene with the activity on a transformant containing a resistant gene or a comparison between the activities on transformants differing in expression level from each other. Also, the screening can be efficiently carried out by measuring the affinity for the protein of the present invention, for example, the activity of inhibiting the binding of radiolabeled-aureobasidin to the protein.

45 [Brief Description of the Drawings]

[Fig. 1]

50 Restriction enzyme map of the genes *spaur1<sup>R</sup>* and *spaur1<sup>S</sup>* regulating aureobasidin sensitivity.

[Fig. 2]

55 Restriction enzyme map of *scaur1<sup>R</sup>* and *scaur1<sup>S</sup>*.

[Fig. 3]

Restriction enzyme map of *scaur2<sup>R</sup>* and *scaur2<sup>S</sup>*.

[Fig. 4]

Structure of a DNA for disrupting the Schizo. pombe spaur1<sup>S</sup> gene.

5 [Fig. 5]

Structure of a DNA for disrupting the S. cerevisiae scaur1<sup>S</sup> gene.

[Fig. 6]

10 Results of the detection of the aur1 gene caaur1 carried by C. albicans by the PCR method.

[Fig. 7]

15 Restriction enzyme map of the caaur1 gene carried by C. albicans.

[Fig. 8]

20 Restriction enzyme map of the caaur2 gene.

[Fig. 9]

Structure of a plasmid YE<sub>p</sub>SCARW3 for expressing the scaur1 gene.

25 [Fig. 10]

Results of the northern hybridization of the spaur1 gene of Schizo. pombe.

[Fig. 11]

30 Results of the detection of the scaur1 protein by using an antibody.

[Fig. 12]

35 Restriction enzyme map of pAR25.

[Examples]

To further illustrate the present invention in greater detail, the following Examples will be given.

40 However it is to be understood that the present invention is not restricted thereto.

Example 1: Cloning of a gene regulating aureobasidin sensitivity originating in fission yeast Schizo. pombe

45 1-a) Separation of aureobasidin-resistant mutant of Schizo. pombe

50 About  $1 \times 10^3$  cells of a Schizo. pombe haploid cell strain JY745 (mating type h<sup>-</sup>, genotype ade6-M210, leu1, ura4-D18) exhibiting a sensitivity to aureobasidin at a concentration of 0.08  $\mu$ g/ml were suspended in 1 ml of a phosphate buffer containing 0.9% NaCl. Then the cells were mutagenized with EMS at a final concentration of 3% at 30 °C for 90 minutes. After neutralizing by adding 8 ml of 5% sodium thiosulfate, the cells thus treated were harvested by centrifugation (2500 r.p.m., 5 minutes), washed twice with 6 ml of physiological saline and then suspended in 2 ml of a YEL medium (3% of glucose, 0.5% of yeast extract). The suspension was incubated at 30 °C for 5 hours under stirring and then spread on a YEA plate (the YEL medium containing 1.5% of agar) containing 5  $\mu$ g/ml of aureobasidin A. After incubating at 30 °C for 3 to 4 days, two or three aureobasidin-resistant colonies were formed per  $1 \times 10^3$  cells. After 55 carrying out the mutagenesis several times, five clone mutants, i.e., THR01, THR04, THR05, THR06 and THR07 were obtained. These mutants were resistant to more than 25  $\mu$ g/ml of aureobasidin A but the same as the parent strain in the sensitivity to cycloheximide and amphotericin B. Therefore it is estimated that they are not mutants having a multiple drug resistance but ones having a resistance specific to

aureobasidin.

1-b) Genetic analysis

5 Each of the above-mentioned resistant strains THR01, THR04, THR05, THR06 and THR07 was crossed with normal cells of Schizo. pombe LH121 strain (mating type  $h^+$ , genotype ade6-M216, ura4-D18) differing in mating type. Diploid cells obtained were examined about the resistance to aureobasidin. Similar to the resistant strains, the five diploids formed by crossing the resistant strains with the normal one were resistant to 25  $\mu$ g/ml of aureobasidin A, thus proving that these resistant mutations were dominant. To perform the 10 tetrad analysis, the above-mentioned diploids were subsequently inoculated on an MEA medium (3% of malt extract, 2.5% of agar) for sporulation and incubated at 25 °C for 2 days. Prior to the meiosis, the diploid cells replicated DNA on the MEA medium and then underwent the meiosis to form ascospores each containing four ascospores of the haploid. These spores were separated with a micromanipulator and allowed to germinate on the YEA plate, followed by the formation of colonies. Then the resistance to 15 aureobasidin of these colonies was examined. Among four spores contained in an ascus, the separation of the sensitivity versus the resistance showed 2 : 2. This result indicates that the aureobasidin resistant mutation was induced by a mutation in single gene. Further, the complementation test was performed in order to confirm whether the resistant genes of the above-mentioned five mutants were identical with each other or not. For example, a mutant of the mating type  $h^+$ , which had been obtained by crossing the mutant 20 THR01 with the LH121 strain in the above tetrad analysis, was crossed with another variant THR04 (mating type  $h^-$ ) on the MEA plate as described above and, after sporulation, the tetrad analysis was carried out. As a result, all of the colonies formed from four ascospores showed resistance to aureobasidin, which indicates that the mutational genes of THR01 and THR04 are the same with each other. Similarly, the five mutants 25 were examined and it was thus found out that all mutations occurred on the same gene. This gene regulating aureobasidin sensitivity is named *spaur1*, the normal gene (sensitive gene) is named *spaur1<sup>S</sup>* and the mutational gene (resistant gene) is named *spaur1<sup>R</sup>*.

1-c) Preparation of genomic library of aureobasidin resistant strain

30 Genomic DNA was extracted and purified from the aureobasidin resistant strain THR01 by the method of P. Philippsen et al. [Methods in Enzymology, 194, 169 - 175 (1991)]. The purified genomic DNA (8  $\mu$ g) was partially digested by treating with 5 U of a restriction enzyme HindIII at 37 °C for 10 minutes, deproteinized with phenol/chloroform and precipitated with ethanol. The partially digested DNA was electrophoresed on a 0.8% agarose gel and DNA in the region of 3 to 15 kb was extracted. The DNA thus 35 obtained was ligated with a yeast-E. coli shuttle vector pAU-PS (2  $\mu$ g) which had been completely digested with HindIII by using a DNA ligation kit (manufactured by Takara Shuzo Co., Ltd.) and then transformed into E. coli HB101. Thus a genomic library of the aureobasidin resistant strain was formed. E. coli containing this genomic library was incubated in 50 ml of an LB medium (1% of bacto tryptone, 0.5% of bacto yeast extract, 0.5% of sodium chloride) containing 100  $\mu$ g/ml of ampicillin and 25  $\mu$ g/ml of tetracycline at 37 °C overnight. 40 Then a plasmid was recovered and purified from the E. coli cells.

1-d) Expression and cloning of aureobasidin resistant gene *spaur1<sup>R</sup>*

The plasmid originating in the genomic library of the aureobasidin resistant strain as prepared above 45 was transformed into a strain Schizo. pombe JY745 by the method of Okazaki et al. [Nucleic Acid Research, 18, 6485 - 6489 (1990)]. The transformed cells were spreaded on a minimum medium SD plate [0.67% of yeast nitrogen base without amino acids (manufactured by Difco), 2% of glucose, 2% of agar] containing 75  $\mu$ g/ml of adenine sulfate and 50  $\mu$ g/ml of leucine. After incubating at 30 °C for 3 to 4 days, the colonies thus formed were replicated onto an SD plate containing 5  $\mu$ g/ml of aureobasidin A, 75  $\mu$ g/ml 50 of adenine sulfate and 50  $\mu$ g/ml of leucine. It is conceivable that a colony propagated on this plate may have the plasmid containing the aureobasidin resistant gene. This colony was inoculated into 5 ml of a liquid SD medium containing 75  $\mu$ g/ml of adenine sulfate and 50  $\mu$ g/ml of leucine. After incubating at 30 °C for 2 days, the plasmid was recovered from the propagated cells by the method of I. Hagan et al. [J. Cell Sci., 91, 587 - 595 (1988)]. Namely, the cells were harvested from the culture (5 ml) by centrifugation and 55 then suspended in 1.5 ml of 50 mM citrate phosphate buffer containing 1.2 M of sorbitol and 2 mg/ml of Zymolyase. Then the suspension was maintained at 37 °C for 60 minutes. The cells were collected by centrifuging at 3,000 r.p.m. for 30 seconds and suspended in 300  $\mu$ l of a TE [10 mM of Tris-HCl, pH 8, 1 mM of EDTA] solution. After adding 35  $\mu$ l of 10% SDS, the mixture was maintained at 65 °C for 5 minutes.

After adding 100  $\mu$ l of 5 M potassium acetate, the mixture was allowed to stand in ice for 30 minutes. Then it was centrifuged at 10.000 r.p.m. at 4°C for 10 minutes and a plasmid DNA was purified from the supernatant by using EASYTRAP™ (manufactured by Takara Shuzo Co., Ltd.).

This plasmid was transformed into *E. coli* HB101 and a plasmid DNA was prepared from *E. coli* colonies formed on an LB medium containing ampicillin and tetracycline. This plasmid, which contained a DNA of 4.5 kb, was named pAR25. Fig. 12 shows the restriction enzyme map of the DNA of 4.5 kb in pAR25. To specify the gene region, HindIII fragments or SacI fragments of various sizes were subcloned into the pAU-PS vector. These DNAs were transformed into normal JY745 cells by the above-mentioned method of Okazaki et al. and the acquisition of aureobasidin resistance was examined. As a result, it is revealed that a HindIII-SacI 2.4 kb DNA fragment contains the spaur1<sup>R</sup> gene. The restriction enzyme map of this DNA segment containing the aureobasidin resistant gene spaur1<sup>R</sup> is shown in Fig. 1. This fragment was cloned into a pUC118 vector (named pUARS2R) and then the DNA nucleotide sequence was identified (SEQ ID No. 1 in Sequence Listing). From this nucleotide sequence, it is revealed that the spaur1<sup>R</sup> gene code for a protein having an amino acid sequence represented by SEQ ID No. 2 in Sequence Listing.

15 1-e) Cloning of aureobasidin sensitive gene spaur1<sup>S</sup>

By the same method as the one employed in the above c), genomic DNA was extracted and purified from normal cells. After partially digesting with HindIII, a genomic library of the normal cells was 20 constructed. An *E. coli* stock containing this library DNA was spread on an LB agar medium containing ampicillin and tetracycline and incubated overnight at 37°C. The colonies thus formed were transferred onto a nylon membrane (Hybond™-N, manufactured by Amersham) and the colony hybridization was performed.

As a probe, the above-mentioned DNA fragment (2.4 kb) obtained by cleaving the spaur1<sup>R</sup> gene with HindIII-SacI and labeled with [ $\alpha$ - $^{32}$ p] dCTP by using a random primer DNA labeling kit (manufactured by 25 Takara Shuzo Co., Ltd.) was used. As the results of screening of  $5 \times 10^4$  colonies, five clones being hybridizable with the probe were obtained. Plasmids were purified from *E. coli* cells of these five clones. As the result of the cleavage with restriction enzymes, it was found out that all of these clones contained the same DNA fragment of 4.5 kb (named pARN1). The restriction enzyme map of the DNA of 4.5 kb in pARN1 30 was identical with that of pAR25 shown in Fig. 10. Therefore, a HindIII-SacI 2.4 kb DNA fragment which was a region containing the spaur1<sup>S</sup> gene was prepared from pARN1. Then it was cloned into the pAU-PS vector and this plasmid was named pSPAR1.

By using this plasmid pSPAR1, a strain *E. coli* JM109 was transformed and the transformant thus obtained was named and designated as *Escherichia coli* JM109 pSPAR1. It has been deposited at National 35 Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4485. This DNA fragment containing the aureobasidin sensitive gene spaur1<sup>S</sup> had the restriction enzyme map shown in Fig. 1 and the DNA nucleotide sequence thereof was the one represented by SEQ ID No. 3 in Sequence Listing. Based on this 40 nucleotide sequence, it has been revealed that the spaur1<sup>S</sup> gene codes for a protein having the amino acid sequence represented by SEQ ID No. 4 in Sequence Listing and, when compared with the resistant gene spaur1<sup>R</sup>, the amino acid at the residue 240 has been changed from glycine into cysteine.

Example 2: Cloning of aureobasidin sensitive genes scaur1 and scaur2 originating in budding yeast *S. cerevisiae*

45 2-a) Separation of aureobasidin resistant mutant of *S. cerevisiae*

A strain *S. cerevisiae* DKD5D (mating type a, genotype leu2-3 112, trp1, his3) having a sensitivity to aureobasidin at a concentration of 0.31  $\mu$ g/ml was mutagenized with EMS in the same manner as the one 50 employed in the case of *Schizo. pombe*. Then resistant mutants were separated on an agar plate of a complete nutritional medium YPD (1% of yeast extract, 2% of polypeptone, 2% of glucose) containing 5  $\mu$ g/ml or 1.5  $\mu$ g/ml of aureobasidin A. After repeating the mutagenesis several times, 34 mutant clones were obtained. These mutants were resistant to more than 25  $\mu$ g/ml of aureobasidin A and estimated as having not a multiple drug resistance mutation but a aureobasidin-specific resistance mutation.

## 2-b) Genetic analysis

Similar to the above-mentioned case of Schizo. pombe, the genetic analysis using the tetrad analysis and the complementation test was performed. As a result, the genes could be classified into two types.

5 These genes regulating aureobasidin sensitivity were named scaur1 and scaur2, the resistant genes isolated from the resistant mutant were named scaur1<sup>R</sup> and scaur2<sup>R</sup>, and the sensitive genes isolated from the sensitive wild-type strain were named scaur1<sup>S</sup> and scaur2<sup>S</sup>, respectively.

The R94A strain had a gene with dominant mutation (scaur1<sup>R</sup>). It has been further clarified that the scaur1 gene is located in the neighborhood of the met14 gene of the eleventh chromosome.

10

2-c) Preparation of genomic library of aureobasidin resistant strain having aureobasidin resistant gene scaur1<sup>R</sup>

15 Genomic DNA was extracted and purified from the aureobasidin resistant strain R94A by the above-mentioned method of P. Philippsen et al. The purified genomic DNA (8 µg) was partially digested by treating with 5 U of a restriction enzyme HindIII at 37 °C for 10 minutes, deproteinized with phenol chloroform and precipitated with ethanol. The partially digested DNA thus obtained was electrophoresed on a 0.8% agarose gel and DNA in the region of 3 to 15 kb was extracted. The DNA thus obtained was ligated with a yeast-E. coli shuttle vector pWH5 (2 µg) which had been completely digested with HindIII by 20 using a DNA ligation kit and then transformed into E. coli HB101. Thus a genomic library was formed. E. coli containing this genomic library was cultured in 50 ml of an LB medium containing ampicillin and tetracycline at 37 °C overnight. Then a plasmid was recovered and purified from the E. coli cells.

25

2-d) Expression and cloning of aureobasidin resistant gene scaur1<sup>R</sup>

25

The above-mentioned genomic library of the R94A strain was transformed into S. cerevisiae SH3328 (mating type  $\alpha$ , genotype ura3-52, his4, thr4, leu2-3 + 112) in accordance with the method of R.H. Schiestl et al. [Current Genetics, 16, 339 - 346 (1989)]. The transformed cells were spread on a minimum medium SD plate [0.67% of yeast nitrogen base without amino acids, 2% of glucose, 2% of agar] containing 25 µg/ml of uracil, 35 µg/ml of histidine and 500 µg/ml of threonine. After incubating at 30 °C for 3 to 4 days, the colonies thus formed were replicated onto a YPD agar plate containing 1.5 µg/ml of aureobasidin A. A colony thus formed was inoculated into 5 ml of a liquid YPD medium. After incubating at 30 °C for 2 days, a plasmid DNA was recovered from the propagated cells by the above-mentioned method of I. Hagan et al. This plasmid was transformed into a yeast again and it was confirmed that the obtained transformant had 35 acquired aureobasidin resistance. This plasmid, which contained a DNA of 3.5 kb, was named pWTCR3. Neither the DNA fragment of 2.0 kb nor the DNA fragment of 1.5 kb obtained by cleaving with HindIII exhibited any aureobasidin resistant activity alone. Thus it is confirmed that the gene is contained in the DNA fragment of 3.5 kb. Fig. 2 shows the restriction enzyme map of this DNA fragment of 3.5 kb containing the aureobasidin resistant gene scaur1<sup>R</sup>. The HindIII fragments of 1.5 kb and 2 kb were each cloned into 40 pUC118, followed by the determination of the DNA nucleotide sequence (SEQ ID No. 5 in Sequence Listing). From this nucleotide sequence, it has been revealed that the scaur1<sup>R</sup> gene codes for a protein having an amino acid sequence represented by SEQ ID No. 6 in Sequence Listing.

45

2-e) Cloning of aureobasidin sensitive gene scaur1<sup>S</sup> corresponding to aureobasidin resistant gene scaur1<sup>R</sup>

45

By the same method as the one employed in the above Example 2-c), genomic DNA was extracted and purified from the parent strain S. cerevisiae DKD5D. After partially digesting with HindIII, the DNA was ligated with pWH5 and transformed into E. coli HB101. Thus a genomic library of the normal cells was formed. An E. coli stock containing this library DNA was spread on an LB agar medium containing ampicillin and tetracycline and incubated overnight at 37 °C. The colonies thus formed were transferred onto a nylon membrane (Hybond™-N) and the colony hybridization was carried out. As a probe, the DNA fragment of 3.5 kb obtained in the above Example 2-d) and labeled with [ $\alpha$ -<sup>32</sup>P] dCTP by using a random primer DNA labeling kit (manufactured by Takara Shuzo Co., Ltd.) was used. As the results of screening of  $2 \times 10^4$  colonies, seven clones being hybridizable with the probe were obtained. Plasmids were purified from E. coli 50 cells of these clones. As the result of the cleavage with restriction enzymes, one of these clones contained a DNA fragment of 3.5 kb. This DNA fragment had the restriction enzyme map of Fig. 2 and thus judged as containing the scaur1<sup>S</sup> gene. The plasmid containing this DNA fragment was named pSCAR1, while E. coli HB101 having this plasmid introduced therein was named and designated as Escherichia coli

HB101 pSCAR1. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4483. The DNA fragment of 3.5 kb obtained by partially digesting pSCAR1 with HindIII was subcloned into pUC118 and the nucleotide sequence thereof was determined (SEQ ID No. 7 in Sequence Listing). A comparison with the resistant gene indicates that the base at the position 852 has been changed from T into A and, due to this replacement, the amino acid has been converted from phenylalanine into tyrosine (SEQ ID No. 8 in Sequence Listing).

2-f) Preparation of genomic library of aureobasidin resistant strain having aureobasidin resistant gene scaur2<sup>R</sup>

A genomic library was prepared from an aureobasidin resistant strain L22-8B by the same method as the one described in Example 2-c). E. coli containing this genomic library was cultured in an LB medium (50 ml) containing ampicillin and tetracycline at 37 °C overnight. Then plasmids were recovered and purified from the E. coli cells.

2-g) Expression and cloning of aureobasidin resistant gene scaur2<sup>R</sup>

The above-mentioned plasmids originating in the genomic library of the L22-8B strain were transformed into S. cerevisiae SH3328 by the above-mentioned method of R.H. Schiestl. From the transformed strains, an aureobasidin resistant strain was isolated. Then a plasmid DNA containing the scaur2<sup>R</sup> gene was recovered from this transformant by the above-mentioned method of I. Hagan et al. This plasmid was transformed into a yeast again and it was confirmed that the transformant had acquired aureobasidin resistance. This plasmid, which contained a DNA of 8.5 kb, was named pSCAR2. Fig. 3 shows the restriction enzyme map of the DNA fragment of 8.5 kb containing this aureobasidin resistant gene scaur2<sup>R</sup>. E. coli HB101 having this plasmid pSCAR2 introduced therein was named and designated as Escherichia coli HB101 pSCAR2. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4484. By using BamHI, EcoRI, HindIII and PstI, DNA fragments of various sizes were prepared and cloned into the pWH5 vector. These plasmids were transformed into S. cerevisiae DKD5D in accordance with the above-mentioned method of R.H. Schiestl et al. Then it was examined whether these transformants had acquired aureobasidin resistance or not. As a result, none of the transformants of the DNA fragments was a resistant one. Thus it has been clarified that the DNA fragment of the full length is necessary for the expression of the aureobasidin resistance.

2-h) Isolation of aureobasidin sensitive gene scaur2<sup>S</sup> corresponding to aureobasidin resistant gene scaur2<sup>R</sup>

An E. coli stock containing the genomic library of Example 2-e) prepared from normal cells of S. cerevisiae DKD5D was spreaded on an LB agar medium containing ampicillin and tetracycline and incubated at 37 °C overnight. The colonies thus formed were transferred onto a nylon membrane (Hybond™ -N) and the colony hybridization was performed. As a probe the DNA fragment of 8.5 kb obtained in the above Example 2-g) and labeled with [ $\alpha$ -<sup>32</sup>P] dCTP by using a random primer DNA labeling kit was used. As the results of screening of  $2 \times 10^4$  colonies, several clones being hybridizable with the probe were obtained. Some of these clones contained a DNA fragment of 4.6 kb while others contained a DNA fragment of 3.9 kb. From the restriction enzyme maps of these DNA fragments, it was found out that these DNA fragments were each a part of the scaur2<sup>S</sup> gene shown in Fig. 3. These DNA fragments were ligated together to thereby give a scaur2<sup>S</sup> fragments shown in Fig. 3. The DNA fragment of 8.5 kb thus obtained was subcloned into pUC118 and then the DNA nucleotide sequence was determined (SEQ ID No. 9 in Sequence Listing). Based on the nucleotide sequence of SEQ ID No. 9 in Sequence Listing, the amino acid sequence represented by SEQ ID No. 10 in Sequence Listing was estimated.

Example 3: Gene disruption test on scaur1<sup>S</sup> and scaur1<sup>S</sup> genes

3-a) Gene disruption test on scaur1<sup>S</sup> gene

In order to examine whether the aureobasidin sensitive gene scaur1<sup>S</sup> is necessary in the cell growth by the gene disruption test, the plasmid pUARS2R prepared in Example 1-d) was first cleaved with BamI and EcoT22I. After eliminating a DNA fragment of 240 bp, the residual DNA fragment was blunted by using a

DNA blunting kit (manufactured by Takara Shuzo Co., Ltd.). Then this DNA was ligated with a DNA containing *ura4<sup>+</sup>* gene of 1.7 kb, which had been obtained by excising from a pUC8ura4 plasmid [Mol. Gen. Genet., 215, 81 - 86 (1988)] by cleaving with HindIII and blunting, to thereby give a plasmid pUARS2RBT22::ura4-1 and another plasmid pUARS2RBT22::ura4-6 in which the *ura4* DNA had been inserted in the opposite direction. Both of these disrupted genes were excised from the vector pUC118 by cleaving with SacI and HindIII and ARS2RBT22::ura4-1 and ARS2RBT22::ura4-6 (Fig. 4), which were *spauro1<sup>S</sup>* DNA fragments containing *ura4<sup>+</sup>*, were purified. The purified DNA fragments were transformed into diploid cells Schizo. pombe C525 (*h<sup>30</sup> h<sup>30</sup>*, *ura4*-D18/*ura4*-D18, *leu1* *leu1*, *ade6*-M210/*ade6*-M216) by the above-mentioned method of Okazaki et al. and then a transformant was screened on an SD agar plate containing leucine. In the transformant thus obtained, one of a pair of *spauro1<sup>S</sup>* genes on the chromosome had been replaced by the disrupted gene ARS2RBT22::ura4-1 or ARS2RBT22::ura4-6 introduced thereinto. These cells were allowed to undergo sporulation on a sporulation medium MEA and subjected to the tetrad analysis. As a result, it was found out that two of the four ascospores formed colonies but the residual two spores formed no colony. That is to say, the spores suffering from the replacement of the normal *spauro1<sup>S</sup>* gene by the disrupted gene ARS2RBT22::ura4-1 were not propagated. It has been thus revealed that the *spauro1<sup>S</sup>* gene is essentially required for the growth of the cells.

3-b) Gene disruption test on *scaur1<sup>S</sup>* gene

The plasmid pSCAR1 prepared in Example 2-e) was partially digested with HindIII to thereby give a DNA fragment of 3.5 kb shown in Fig. 2. This DNA fragment was cloned into the HindIII site of pUC119 and the obtained product was named pSCAR3. The obtained pSCAR3 was cleaved with StuI and EcoT22I. After eliminating a DNA fragment of 0.3 kb, the obtained DNA was ligated with a DNA fragment (1.1 kb) of URA3 gene which had been obtained by cleaving a plasmid pYEura3 (manufactured by Clontech Laboratories, Inc.) with HindIII and EcoRI and blunting. Thus a plasmid pUSCAR3.ST22::URA3<sup>+</sup> and another plasmid pUSCAR3.ST22::URA3A, in which the URA3 gene had been inserted in the opposite direction, were obtained. These disrupted gene were excised in the EcoRI site in the *scaur1<sup>S</sup>* gene and the EcoRI site in the pUC119 vector by cleaving with EcoRI. The *scaur1<sup>S</sup>* DNA fragments containing URA3, SCAR3.ST22::URA3<sup>+</sup> and SCAR3.ST22::URA3A (Fig. 5), were purified. The purified DNA fragments were transformed into diploid cells of S. cerevisiae AOD1 (mating type *a/α*, genotype *ura3*-52/*ura3*-52, *leu2*-3 112-*leu2*-3 112, *trp1*/*TRP1*, *thr4*/*THR4*, *his4*/*HIS4*) by the above-mentioned method of R.H. Schiestl and transformants were screened on an SD agar plate containing leucine. The transformants thus obtained were allowed to undergo sporulation on a sporulation medium SP (1% of potassium acetate, 2% of agar) and subjected to the tetrad analysis. As a result, it was found out that two of the four ascospores underwent germination and formed colonies but the residual two spores did not undergo colony formation. That is to say, the spores suffering from the replacement of the *scaur1<sup>S</sup>* gene by the disrupted gene were not propagated. It has been thus revealed that the *scaur1<sup>S</sup>* gene is essentially required for the growth of the cells.

40 Example 4: Examination on the expression of aureobasidin sensitive gene *spauro1* by northern hybridization

From a normal strain or a resistant strain of Schizo. pombe, the whole RNAs were extracted and purified by the method of R. Jensen et al. [Proc. Natl. Acad. Sci. USA, 80, 3035 - 3039 (1983)]. Further, poly(A)<sup>+</sup>RNA was purified by using Oligotex<sup>TM</sup>-dT30 (manufactured by Takara Shuzo Co., Ltd.). The purified poly(A)<sup>+</sup>RNA (2.5  $\mu$ g) was separated by the electrophoresis on a 1.2% agarose gel containing formaldehyde and transferred onto a nylon membrane (Hybond<sup>TM</sup>-N). After immobilizing, the hybridization was performed with the use of a HindIII-SacI fragment (2 kb) of the *spauro1<sup>R</sup>* gene labeled with [ $\alpha$ -<sup>32</sup>P]dCTP as a probe. As a result, both of the normal cells and the resistant cells showed a band of the same amount of about 2 kb. In both cases, this amount underwent no change in the logarithmic growth phase and the stationary phase (Fig. 10). Fig. 10 is an autoradiogram showing the results of the northern hybridization wherein mRNAs obtained from cells of a sensitive strain of Schizo. pombe in the logarithmic growth phase (lane 1), cells of a resistant strain in the logarithmic growth phase (lane 2), cells of the sensitive strain in the stationary phase (lane 3) and cells of the resistant strain in the stationary phase (lane 4) are electrophoresed on a 1.2% agarose gel containing formaldehyde.

Example 5: Determination of the activity of *scaur1<sup>S</sup>* gene5-a) Construction of plasmid YE<sub>p</sub>SCARW3 (Fig. 9) and YE<sub>p</sub>SCARW1

5 The plasmid pSCAR1 prepared in Example 2-e) was cleaved with HindIII and a fragment of 2 kb containing the whole ORF was excised. This fragment was inserted into the HindIII site of a expression-plasmid YE<sub>p</sub>52 having a promoter Gal10, the expression of which was induced by galactose in a medium. The plasmid having the *scaur1<sup>S</sup>* gene which had been inserted in such a direction as to be normally transcribed by the promoter Gal10 was named YE<sub>p</sub>SCARW3. Fig. 9 shows the structure of this plasmid.  
10 Further, the plasmid having the *scaur1<sup>S</sup>* gene inserted in the opposite direction was named YE<sub>p</sub>SCARW1.

5-b) Transformation by plasmids YE<sub>p</sub>SCARW3 and YE<sub>p</sub>SCARW1

15 By using 5  $\mu$ g portions of the plasmids YE<sub>p</sub>SCARW3 and YE<sub>p</sub>SCARW1, the diploid S. cerevisiae cells with the disrupted *scaur1<sup>S</sup>* gene prepared in Example 3-b) were transformed. Then transformants were screened on an SD agar plate. These transformants were allowed to undergo sporulation on an SP medium and then subjected to the tetrad analysis. When the expression of the *scaur1<sup>S</sup>* gene was induced by using a YPGal medium (1% of yeast extract, 2% of polypeptone, 2% of galactose), the ascospores formed from the diploid cells transformed by YE<sub>p</sub>SCARW3 all underwent germination while two of the four ascospores formed from the diploid cells transformed by YE<sub>p</sub>SCARW1 underwent germination but not the remaining two. It is thus conceivable that the cells with the disrupted *scaur1<sup>S</sup>* gene have reverted to the normal state by introducing YE<sub>p</sub>SCARW3 containing the *scaur1<sup>S</sup>* gene into these cells. Accordingly, the use of these cells with the disrupted *scaur1<sup>S</sup>* gene as a host makes it possible to determine the activity of normal aur1-analogous genes carried by other organisms.

25 Example 6: Confirmation and cloning of aur1 and aur2 genes (caaur1, caaur2) carried by C. albicans

## 6-a) Detection of aur1 gene by the PCR method

30 Poly(A)<sup>-</sup>RNA was extracted and purified from an aureobasidin sensitive strain C. albicans TIMM0136 by the same method as the one employed in Example 4. By using the poly(A)<sup>+</sup>RNA (5  $\mu$ g) as a template, a double-stranded cDNA was synthesized on a cDNA synthesizing system Plus (manufactured by Amersham) with the use of an oligo(dT) primer. Mixed primers for PCR corresponding to amino acid sequence regions being common to the amino acid sequences of S. cerevisiae and Schizo. pombe were synthesized on a 35 DNA synthesizer and purified. That is to say, a primer of SEQ ID No. 11 in Sequence Listing corresponding to the region of amino acids at the 184- to 192-positions of SEQ ID No. 4 in sequence Listing of Schizo. pombe (from the 184- to 192-positions of SEQ ID No. 8 in Sequence Listing of S. cerevisiae) and another primer of SEQ ID No. 12 in Sequence Listing corresponding to the region of amino acids from the 289- to 40 298-positions of Schizo. pombe (from the 289- to 298-positions of SEQ ID No. 8 in Sequence Listing of S. cerevisiae) were employed.

45 PCR was performed by using these primers and the above-mentioned cDNA as a template by repeating a cycle comprising treatment at 94 °C for 30 seconds, one at 48 °C for 1 minute and one at 72 °C for 2 minutes 25 times. As a result, a DNA (about 350 bp) being almost the same as S. cerevisiae and Schizo. pombe in length was amplified (Fig. 6). Fig. 6 shows a pattern obtained by carrying out PCR with the use of 50 cDNA of C. albicans (lane 1), cDNA of S. cerevisiae (lane 2) and cDNA of Schizo. pombe (lane 3) as a template, electrophoresing each PCR product on an agarose gel and staining with ethidium bromide.

6-b) Cloning of aur1 gene (caaur1) of C. albicans

50 (i) Genomic DNA was extracted and purified from a strain C. albicans TIMM0136 by the same method as the one described in Example 1-c). After partially digesting with HindIII, the DNA fragment was ligated with a TraplexII9 vector which had been completely digested with HindIII and transformed into E. coli HB101. Thus a genomic library of C. albicans was prepared. From this library, a DNA fragment of 4.5 kb containing the aur1 gene of C. albicans was cloned by using the DNA fragment of C. albicans obtained by the PCR described in Example 6-a), which had been labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by using a random 55 primer DNA labeling kit (manufactured by Takara Shuzo Co., Ltd.), as a probe. This DNA fragment had a restriction enzyme map shown in Fig. 7 and the DNA nucleotide sequence thereof is represented by SEQ ID No. 13 in Sequence Listing. Based on this nucleotide sequence, it was estimated that the caaur1

5 gene coded for a protein having the amino acid sequence represented by SEQ ID No. 14 in Sequence Listing. When compared with the scaur1<sup>S</sup> protein, a homology of as high as 53% was observed. A TraplexII9 vector having this caaur1 gene integrated therein was named pCAAR1, while E. coli HB101 transformed by this plasmid was named and designated as Escherichia coli HB101/pCAAR1. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4482.

10 Next, pCAAR1 was treated with HindIII to thereby give caaur1 of 4.5 kb. Further, it was integrated into pTV118 which had been completely digested with HindIII to thereby prepare a plasmid for expressing caaur1. This plasmid was named pTCAAR1.

(ii) Genomic DNA was extracted and purified from a strain C. albicans TIMM1768 [The journal of Antibiotics, 46, 1414-1420(1993)] by the same method as the one described in example 1-c). After 15 partially digesting with Hind III, the DNA fragment was ligated with a pUC118 vector which had been completely digested with Hind III and transformed into E. coli HB101. Thus a genomic library of C. albicans TIMM1768 was prepared. From this library, a DNA fragment of 4.5 kb containing the aur1 gene of C. albicans TIMM1768 was cloned by the colony hybridization with the same probe as that described 20 in Example 6-b)-(i). This DNA fragment had the same restriction enzyme map as that shown in Fig. 7. Next, a part of the DNA sequence containing a ORF in this DNA fragment was determined. The DNA nucleotide sequence thereof is represented by SEQ ID No. 21 in Sequence Listing. Based on this 25 nucleotide sequence, it was estimated that this gene coded for a protein having the amino acid sequence represented by SEQ ID No 22 in Sequence Listing. When the amino acid sequence of the caaur1 protein C. albicans TIMM1768 was compared with that of the caaur1 protein of C. albicans TIMM0136, the amino acid sequences of the 1- to 381-positions and the 383- to 423-positions and the 425- to 471-positions of caaur1 protein (SEQ ID No. 14 in Sequence Listing) in C. albicans TIMM0136 were identical 30 with the amino acid sequences of the 2- to 382-positions and the 384- to 424-positions and the 426- to 472-positions, respectively, of caaur1 protein (SEQ ID No. 22 in Sequence Listing) in C. albicans TIMM1768.

However, serines at the 382- and 424-positions of SEQ ID No. 14 in Sequence Listing were replaced with prolines at the 383- and 425-positions of SEQ ID No. 22 in Sequence Listing.

30 6-c) Cloning of aur2 gene (caaur2) of C. albicans

Genomic DNA of a strain C. albicans TIMM0136 was digested with BamHI and ligated with a pTV118 35 vector which had been completely digested with BamHI. Then it was transformed into E. coli HB101 to thereby prepare a genomic library of C. albicans. On the other hand, the DNA fragment containing the scaur2<sup>S</sup> gene obtained in Example 2-h) was cleaved with HindIII and PstI to thereby give a DNA fragment of 1.2 kb. This DNA fragment was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by using a random primer DNA labeling kit. By 40 using this labeled DNA fragment as a probe, the above-mentioned C. albicans genomic library was screened by the colony hybridization. Thus a plasmid containing a DNA fragment of 8.3 kb was obtained. A part of the DNA sequence upstream of the BamHI site of this DNA fragment was determined (SEQ ID No. 15 in Sequence Listing). Based on this sequence, an amino acid sequence represented by SEQ ID No. 16 45 in Sequence Listing was estimated. It corresponded to the amino acid sequence of the 1230- to 1309-positions of the amino acid sequence of the scaur2 gene (SEQ ID No. 10), having a homology of as high as 77%. Since this DNA fragment lacked a part of the C-end, the genomic library prepared in Example 6-b) was further screened by using this DNA fragment as a probe. Thus a DNA fragment of 6.5 kb having the C-terminal part was obtained. Fig. 8 shows the restriction enzyme map of the DNA region containing the caaur2 gene thus clarified.

50 A pTV118 vector having the above-mentioned caaur2 gene of 8.3 kb integrated therein was named pCAAR2N, while E. coli HB101 transformed by this plasmid was named and designated as Escherichia coli HB101/pCAAR2N. This strain has been deposited at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession number FERM BP-4481.

Example 7: Preparation of antibody against protein coded for by *scaur1<sup>S</sup>* gene and staining of *S. cerevisiae* cells and detection of said protein by using this antibody.

7-a) Preparation of antibody

5 SCAR1-1 (SEQ ID No. 19 in Sequence Listing) comprising a peptide corresponding to the amino acids at the residue 103 to 113 in the amino acid sequence of SEQ ID No. 8 in Sequence Listing having cysteine added to the N-end thereof and SCAR1-2 (SEQ ID No. 20 in Sequence Listing) comprising a peptide corresponding to the amino acids at the residue 331 to 348 in the amino acid sequence of SEQ ID No. 8  
10 having cysteine added to the N-end thereof were synthesized by the Fmoc solid phase synthesis method and purified by reverse phase HPLC. Thus 10 mg portions of these peptides were obtained. To the N-terminal cysteine of each of these synthetic peptides, KLH was bound as a carrier protein. By using this binding product as an antigen, a rabbit was immunized and an antiserum was obtained. This antiserum was further purified on an affinity column prepared by binding the synthetic peptide employed as the antigen to  
15 an agarose gel. This a polyclonal antibody being specific for the synthetic peptide was prepared.

7-b) Staining of *S. cerevisiae* cells with antibody

20 A strain *S. cerevisiae* ATCC 9763 was cultured in a YNBG medium [0.67% of yeast nitrogen base (manufactured by Difco), 2% of glucose] to thereby give a suspension of a concentration of  $3 \times 10^7$  cells/ml. To 1 ml of this cell suspension were added 0.11 ml of a 1 M phosphate buffer (pH 6.5) and 0.17 ml of 37% formaldehyde. After slowly stirring at room temperature for 1 hour, the cells were harvested by centrifugation and then suspended in 20 ml of an SS buffer (1 M of sorbitol, 0.2 % of  $\beta$ -mercaptoethanol, 0.1 M phosphate buffer, pH 7.5) containing 20  $\mu$ g/ml of Zymolyase 20T. After treating at 30 °C for 1 hour, 25 the cells were harvested, washed with the SS buffer, suspended in 1 ml of the SS buffer containing 0.1% of Triton X-100 and then allowed to stand for 10 minutes. This cell suspension was placed on a slide glass which had been coated with poly(L-lysine) and allowed to stand for 10 minutes. Next, a PBS solution containing 1% of albumin (BSA) was dropped thereinto. After allowing to stand at room temperature for 15 minutes, the excessive liquid was removed and then a PBS solution containing BSA containing 0.02 mg/ml of the antiSCAR1-1 antibody was dropped thereinto. After allowing to stand at room temperature for 60 minutes and washing with PBS containing BSA three times, antirabbit IgG antibody labeled with FITC (antibody concentration 0.02 mg/ml) was layered over and allowed to stand at room temperature for 1 hour. 30 After washing with a PBS solution containing BSA, a small amount of a mounting solution, which was a solution prepared by dissolving 0.1 g of p-phenylenediamine in 10 ml of CBS (150 mM of NaCl, 50 mM of CHES, pH 9.5), adjusting the pH value to 9.0 with 10 N NaOH and further adding 90 ml of glycerol, was layered over. Then a cover glass was placed thereon to thereby give a specimen. This specimen was observed under a fluorescence microscope to thereby examine the intracellular distribution of the *scaur1* protein. As a result, it was found out that this protein was distributed all over the cells.

40 7-c) Detection of protein coded for by *scaur1* gene by using antibody

The plasmid YEpSCARW3 prepared in Example 5-a) was introduced into a normal haploid *S. cerevisiae* SH3328 to thereby give a transformant. This transformant was cultured in a YPGal medium or a YPD medium and the cells were harvested by centrifugation. The cells thus obtained were suspended in a buffer (1% of Triton X-100, 1% of SDS, 20 mM of Tris-HCl, pH 7.9, 10 mM of EDTA, 1 mM of DTT, 1 mM of PMSF). Further, glass beads were added thereto to disrupt the cells by vigorous vortex. Then an SDS loading solution was added thereto and the protein was denatured by treating at 95 °C for 5 minutes. After centrifuging, a part of the obtained supernatant was subjected to SDS-PAGE and the protein thus separated was transferred onto an Immobilon membrane (manufactured by MILLIPORE). This Immobilon membrane 45 was treated with Block Ace (manufactured by Dainippon Pharmaceutical Co., Ltd.). Then the antiSCAR1-2 antibody prepared in 7-a) was reacted therewith as a primary antibody. After washing, antirabbit IgG antibody labeled with peroxidase was reacted therewith as a secondary antibody and the mixture was thoroughly washed. Next, it was color-developed with diaminobenzidine and a band of the *scaur1* protein 50 was detected. Fig. 11 shows the results.

55 Fig. 11 shows the results of the detection of the protein prepared from the cells incubated in the YPD medium (lane 1) and the protein prepared from the cells incubated in the YPGal medium (lane 2), each subjected to SDS-PAGE, by using the antiSCAR1-2 antibody. The cells incubated in the YPGal medium, of which *scaur1* gene had been induced, showed a specific band.

[Effects of the Invention]

According to the present invention, a novel protein regulating aureobasidin sensitivity and a gene coding for the protein, i.e., a gene regulating aureobasidin sensitivity are provided. These substances are useful in the diagnosis and treatment for diseases caused by organisms having the above-mentioned gene, such as mycoses. The present invention further provides an antisense DNA and an antisense RNA of this gene, a nucleic acid probe being hybridizable with the gene, a process for detecting the gene by using this nucleic acid probe, a process for producing a protein regulating aureobasidin sensitivity by using a transformant having the gene introduced thereinto, an antibody for the protein and a process for detecting the protein by using this antibody. They are also useful in the diagnosis and treatment of diseases including mycoses.

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## Sequence Listing

SEQ ID NO : 1

SEQUENCE LENGTH : 2385

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

## SEQUENCE DESCRIPTION :

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TAATAAACTA ATTATATTAT	ATATAATTAG CAATTTATA	AAAAAAATAA AAAAATAGCC	120
CTGATTGCTG GCAACTGTGA	GCTAACATT GGTTAACCGG	TCCATCTTT TTTAAATATT	180
TTACATCGCT ACTTTTAAGT	GCTTGACACT TGCATTTAAT	AGCTACTTTC TTTCCCTCAT	240
AAAAATTCTT TTTTTTCCT	TTAGTTTCC GGTTAATTCC	TTACGAAATT TTTTCGTAC	300
GCTTCCCTTT TTTACTCTGA	TAATTCTTG AAGCAATGTC	TGCTCTTCG ACCTTAAAAA	360
AGCGCCCTTGC TGCCTGTAAC	CGAGCATCCC AATACAAGTT	GGAAACAAGC TTAAACCCCTA	420
TGCCTACATT TCGTTTCTGA	CGCAATACGA AATGGTCATG	GACACATTG CAATATGTGT	480
TTCTAGCAGG TAATTGATT	TTTGCTTGTAA TTGTCATTGA	ATCTCCTGGA TTCTGGGGGA	540
AATTGGCAT TGCCTGTCTT	TTGGCCATTG CGTTGACCGT	TCCTTTAACCA CGCCAAATTT	600
TTTTTCCTGC CATTGTTATC	ATCACCTGGG CAATTTTATT	TTACTCTTGT AGGTTTATTC	660
CAGAACCGCTG GCGTCCACCC	ATATGGGTTG CTGTTTACCC	CACACTTGAA AATATTCTTT	720
ATGGCTCTAA TCTTTCTAGT	CTTCTCTCGA AAACCACGCA	TAGCATCCTT GATATTGG	780
CCTGGGTTCC ATATGGAGTC	ATGCATTATT CGGCTCCTT	TATCATTCA TTTATTCTTT	840
TCATCTTGC ACCTCCTGGA	ACTCTTCCAG TTTGGGCTCG	AACTTTGGT TATATGAATT	900
TATTTGGTGT TCTTATCCAA	ATGGCTTCC CCTGTTCTCC	TCCTGGTAT GAAAATATGT	960
ATGGTTAGA ACCTGCCACG	TATGCAGTAC GTGGCTCTCC	TGGTGGATTG GCGCGTATTG	1020
ATGCTCTCTT CGGCACTAGC	ATTTACACTG ATTGTTTTC	TAACTCTCCG GTTGTAAAAA	1080
GTGCCTTCC ATCTCTTCAC	GCTGGATGGG CCATGCTGGA	AGCACTTTTC CTTTCGCATG	1140
TGTTTCCCTCG ATACCGCTTC	TGCTTTATG GATATGTTCT	ATGGCTTGC TGGTGTACTA	1200
TGTACCTTAC CCACCACTAC	TTTGTAGATT TGGTCGGCGG	TATGTGTTA GCTATTATAT	1260

	GCTTCGTTT	TGCTCAAAAG	CTACGCCCTCC	CACAGTTGCA	AACTGGTAAA	ATCCTTCGTT	1320
5	GGAAATACGA	GTTTGTATC	CACGGTCATG	GTCTTCCGA	AAAAACCAGC	AACTCCTTGG	1380
	CTCGTACCGG	CAGCCCATAAC	TTACTTGAA	GGGATTCTT	TACTCAAAAC	CCTAATGCAG	1440
	TAGCCTTCAT	GAGTGGTCTT	AACAATATGG	AACTTGCTAA	CACCGATCAT	GAATGGTCCG	1500
10	TGGGTTCATC	ATCACCTGAG	CCGTTACCTA	GTCCTGCTGC	TGATTGATT	GATCGTCCTG	1560
	CCAGTACAC	TTCCTCCATC	TTTGATGCAA	GTCATCTTCC	TTAAATCAAC	GTGCTTAAAG	1620
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15	TGGAATGATG	TTGTTCATTG	TGTTTTTAA	CTGTTAATCT	GACATCCATT	GAGTCATTCT	1740
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20	ACCGCTTTG	GTCTCCACTT	CCTTTTCGAA	ACTCTTAACA	GCGATTAGGC	CGGGTATCTT	1860
	CCAGTGTGAT	GTATAGGTAT	TTGTCGTTT	TTTATCATT	CCGTTAATAA	AGAACTCTT	1920
25	TATCCAGCTT	CTTACACTGT	CAACTGTTGT	GAAAGGAACA	CATTAGAAT	TTCATTTCC	1980
	TTATTTGTTG	TGATTAAAT	CGTTTGACAT	AATTTAAAT	TTGGTTGAA	ATGTGTGTGA	2040
	GAAGGCTTGT	TTTATTTCATT	TAGTTTATTG	CTTGTGGCA	CGAAAATCCA	GAACGGAGCA	2100
30	TTAATGTAAT	CCTTTTTAT	TCTGTAAAGC	GTTTTATAC	AAATGTTGGT	TATACGTTTC	2160
	TAAAATAAGA	ATATTGTTAT	AATAATATAG	TTTTTCTAT	CATTGTTAC	ACACACTAAA	2220
	GAGACATTAA	GGATAAGCAA	ATGTGTTAAA	ATGATAATAT	ATTTGGAAA	CATTATATAA	2280
	GAAATTAAGC	AGCTTGACT	AACTACATT	TTGTTTTT	CCTAAGCAA	ACTGTATAGT	2340
	TATACACGCG	AGCTGTATT	ACTTCCATTG	TAGTGACTTG	AGCTC		2385

35 SEQ ID NO : 2

SEQUENCE LENGTH : 422

SEQUENCE TYPE : amino acid

40 STRANDEDNESS : single

TOPOLOGY : linear

45 MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ser Ala Leu Ser Thr Leu Lys Lys Arg Leu Ala Ala Cys Asn

50 1 5 10 15

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Arg Ala Ser Gln Tyr Lys Leu Glu Thr Ser Leu Asn Pro Met Pro  
 20 25 30  
 5 Thr Phe Arg Leu Leu Arg Asn Thr Lys Trp Ser Trp Thr His Leu  
 35 40 45  
 10 Gln Tyr Val Phe Leu Ala Gly Asn Leu Ile Phe Ala Cys Ile Val  
 50 55 60  
 15 Ile Glu Ser Pro Gly Phe Trp Gly Lys Phe Gly Ile Ala Cys Leu  
 65 70 75  
 20 Leu Ala Ile Ala Leu Thr Val Pro Leu Thr Arg Gln Ile Phe Phe  
 80 85 90  
 25 Pro Ala Ile Val Ile Ile Thr Trp Ala Ile Leu Phe Tyr Ser Cys  
 95 100 105  
 Arg Phe Ile Pro Glu Arg Trp Arg Pro Pro Ile Trp Val Arg Val  
 110 115 120  
 30 Leu Pro Thr Leu Glu Asn Ile Leu Tyr Gly Ser Asn Leu Ser Ser  
 125 130 135  
 35 Leu Leu Ser Lys Thr Thr His Ser Ile Leu Asp Ile Leu Ala Trp  
 140 145 150  
 Val Pro Tyr Gly Val Met His Tyr Ser Ala Pro Phe Ile Ile Ser  
 155 160 165  
 40 Phe Ile Leu Phe Ile Phe Ala Pro Pro Gly Thr Leu Pro Val Trp  
 170 175 180  
 Ala Arg Thr Phe Gly Tyr Met Asn Leu Phe Gly Val Leu Ile Gln  
 185 190 195  
 45 Met Ala Phe Pro Cys Ser Pro Pro Trp Tyr Glu Asn Met Tyr Gly  
 200 205 210  
 Leu Glu Pro Ala Thr Tyr Ala Val Arg Gly Ser Pro Gly Gly Leu  
 215 220 225  
 50 Ala Arg Ile Asp Ala Leu Phe Gly Thr Ser Ile Tyr Thr Asp Cys

## EP 0 644 262 A2

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5	Phe Ser Asn Ser Pro Val Val Phe Gly Ala Phe Pro Ser Leu His		
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	Ala Gly Trp Ala Met Leu Glu Ala Leu Phe Leu Ser His Val Phe		
10	260	265	270
	Pro Arg Tyr Arg Phe Cys Phe Tyr Gly Tyr Val Leu Trp Leu Cys		
	275	280	285
15	Trp Cys Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Val		
	290	295	300
	Gly Gly Met Cys Leu Ala Ile Ile Cys Phe Val Phe Ala Gin Lys		
20	305	310	315
	Leu Arg Leu Pro Gin Leu Gin Thr Gly Lys Ile Leu Arg Trp Glu		
	320	325	330
25	Tyr Glu Phe Val Ile His Gly His Gly Leu Ser Glu Lys Thr Ser		
	335	340	345
	Asn Ser Leu Ala Arg Thr Gly Ser Pro Tyr Leu Leu Gly Arg Asp		
30	350	355	360
	Ser Phe Thr Gin Asn Pro Asn Ala Val Ala Phe Met Ser Gly Leu		
	365	370	375
35	Asn Asn Met Glu Leu Ala Asn Thr Asp His Glu Trp Ser Val Gly		
	380	385	390
	Ser Ser Ser Pro Glu Pro Leu Pro Ser Pro Ala Ala Asp Leu Ile		
40	395	400	405
	Asp Arg Pro Ala Ser Thr Thr Ser Ser Ile Phe Asp Ala Ser His		
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45	Leu Pro		

SEQ ID NO : 3

50 SEQUENCE LENGTH : 2385

55

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

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 TAATAAACTA ATTATATTAT ATATAATTAG CAATTTATA AAAAAAATAA AAAAATAGCC 120  
 15 CTGATTGCTG GCAACTGTGA GCTGAACATT GGTTAACCGG TCCATCTTT TTTAAATATT 180  
 TTACATCGCT ACTTTAAGT GCTTGACACT TGCATTTAAT AGCTACTTTC TTTCTTCAT 240  
 AAAAATTCCCT TTTTTTCCT TTAGTTTCC GGTTAATTCC TTACGAAATT TTTTCGTAC 300  
 20 GCTTCCCTT TTTACTCTGA TAATTCTTC AAGCAATGTC TGCTCTTCG ACCTTAAAAA 360  
 AGCCCTTGC TCGGTGTAAC CGAGCATCCC AATACAAGTT GGAAACAAGC TTAAACCCCTA 420  
 TGCCTACATT TCGTTTGCTA CGCAATACGA AATGGTCATG GACACATTG CAATATGTGT 480  
 25 TTCTAGCAGG TAATTGATT TTTGCTTGT A TTGTCATTGA ATCTCCTGG A TTCTGGGGGA 540  
 AATTGGCAT TGCCTGTCTT TTGGCCATTG CGTTGACCGT TCCTTAAACA CGCCAAATT 600  
 TTTTCCTGC CATTGTTATC ATCACCTGGG CAATTTATT TTACTCTTGT AGGTTTATTC 660  
 30 CAGAACGCTG GCGTCCACCC ATATGGTTC GTGTTTACCC CACACTGAA AATATTCTT 720  
 ATGGCTCTAA TCTTTCTAGT CTTCTCTGA AAACCACCGA TAGCATCCTT GATATTGG 780  
 CCTGGGTTCC ATATGGAGTC ATGCATTATT CGGCTCCTT TATCATTCA TTTATTCTT 840  
 35 TCATCTTGC ACCTCCTGG A CTCTTCCAG TTTGGGCTCG AACTTTGGT TATATGAATT 900  
 TATTGGTGT TCTTATCCAA ATGGCTTCC CCTGTTCTCC TCCTTGGTAT GAAAATATGT 960  
 ATGGTTAGA ACCTGCCACG TATGCAGTAC GTGGCTCTCC TGGTGGATTG GCGCGTATTG 1020  
 40 ATGCCTCTT CCGCACTAGC ATTTACACTG ATGGTTTTC TAACTCTCCG GTTGTGTTG 1080  
 GTGCCCTTCC ATCTCTTAC GCTGGATGGG CCATGCTGG A GCACTTTTC CTTTCCATG 1140  
 TGTTCCTCG ATACCGCTTC TGCTTTATG GATATGTTCT ATGGCTTGC TGGTGTACTA 1200  
 45 TGTACCTTAC CCACCACTAC TTTGTAGATT TGGTCGGCGG TATGTGTTA GCTATTATAT 1260  
 GCTTCGTTTT TGCTCAAAAG CTACGCCCTCC CACAGTTGCA AACTGGTAAA ATCCTTCGTT 1320  
 50 GGGAAATACGA GTTTGTTATC CACGGTCATG GTCTTCCGA AAAAACCAGC AACTCCTTGG 1380  
 CTCGTACCGG CAGCCCATAAC TTACTTGGAA GGGATTCTT TACTCAAAAC CCTAATGCAG 1440

5 TAGCCTTCAT GAGTGGTCTT AACAAATATGG AACTTGCTAA CACCGATCAT GAATGGTCCG 1500  
 TGGGTTCATC ATCACCTGAG CCGTTACCTA GTCCTGCTGC TGATTTGATT GATCGTCCTG 1560  
 CCAGTACCAAC TTCTCCATC TTTGATGCAA GTCATCTTCC TTAAATCAAC GTGCTTAAG 1620  
 AATATATTTTC CAAAAGCTAC ATGATACATT GACTAGAATC GGTTTGATTTC ATAGTGGTAT 1680  
 TGGAAATGATG TTGTTCATTG TGTTTTTAA CTGTTAATCT GACATCCATT GAGTCATTCT 1740  
 10 TTACAATTTG TAAAATTAAT TTGTATCACT AATTTGAAG GAAGCTATTT TGGTATTAAT 1800  
 ACCGCTTTG GTCTCCACTT CCTTTTCGAA ACTCTTAACA GCGATTAGGC CGGGTATCTT 1860  
 15 CCAGTGTGAT GTATAGGTAT TTGTCGTTTT TTTATCATT CCGTTAATAA AGAACTCTTT 1920  
 TATCCAGCTT CTTACACTGT CAACTGTTGT GAAAGGAACA CATTAGAAT TTCATTTCC 1980  
 20 TTATTTGTTG TGATTTAAAT CGTTGACAT AATTTAAAT TTGTTTGAA ATGTGTGTGA 2040  
 GAAGGCTTGT TTTATTCACT TAGTTTATTG CTTGTTGCA CGAAAATCCA GAACGGAGCA 2100  
 TAAATGTAAT CCTTTTTAT TCTGAAAGC GTTTTTATAC AAATGTTGGT TATACGTTTC 2160  
 25 TAAAATAAGA ATATTGTTAT AATAATATAG TTTTTCTAT CATTGTTAC ACACACTAAA 2220  
 GAGACATTAAGGATAAGCAA ATGTGTTAAA ATGATAATAT ATTTGGAAA CATTATAAAA 2280  
 GAAATTAAGC AGCTTGACT AACTACATTT TTGTTTTTT CCTAAGCAAA ACTGTATAGT 2340  
 TATACACCGCG AGCTGTATTC ACTTCCATTG TAGTGACTTG AGCTC 2385

30

SEQ ID NO : 4

SEQUENCE LENGTH : 422

35

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

40

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ser Ala Leu Ser Thr Leu Lys Lys Arg Leu Ala Ala Cys Asn

45

1 5 10 15

Arg Ala Ser Gln Tyr Lys Leu Glu Thr Ser Leu Asn Pro Met Pro

50

20 25 30

Thr Phe Arg Leu Leu Arg Asn Thr Lys Trp Ser Trp Thr His Leu

55

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	35	40	45
5	Gln Tyr Val Phe Leu Ala Gly Asn Leu Ile Phe Ala Cys Ile Val		
	50	55	60
10	Ile Glu Ser Pro Gly Phe Trp Gly Lys Phe Gly Ile Ala Cys Leu		
	65	70	75
15	Leu Ala Ile Ala Leu Thr Val Pro Leu Thr Arg Gln Ile Phe Phe		
	80	85	90
20	Pro Ala Ile Val Ile Ile Thr Trp Ala Ile Leu Phe Tyr Ser Cys		
	95	100	105
25	Arg Phe Ile Pro Glu Arg Trp Arg Pro Pro Ile Trp Val Arg Val		
	110	115	120
30	Leu Pro Thr Leu Glu Asn Ile Leu Tyr Gly Ser Asn Leu Ser Ser		
	125	130	135
35	Leu Leu Ser Lys Thr Thr His Ser Ile Leu Asp Ile Leu Ala Trp		
	140	145	150
40	Val Pro Tyr Gly Val Met His Tyr Ser Ala Pro Phe Ile Ile Ser		
	155	160	165
45	Phe Ile Leu Phe Ile Phe Ala Pro Pro Gly Thr Leu Pro Val Trp		
	170	175	180
50	Ala Arg Thr Phe Gly Tyr Met Asn Leu Phe Gly Val Leu Ile Gln		
	185	190	195
55	Met Ala Phe Pro Cys Ser Pro Pro Trp Tyr Glu Asn Met Tyr Gly		
	200	205	210
60	Leu Glu Pro Ala Thr Tyr Ala Val Arg Gly Ser Pro Gly Gly Leu		
	215	220	225
65	Ala Arg Ile Asp Ala Leu Phe Gly Thr Ser Ile Tyr Thr Asp Gly		
	230	235	240
70	Phe Ser Asn Ser Pro Val Val Phe Gly Ala Phe Pro Ser Leu His		
	245	250	255

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Ala Gly Trp Ala Met Leu Glu Ala Leu Phe Leu Ser His Val Phe  
260 265 270  
5 Pro Arg Tyr Arg Phe Cys Phe Tyr Gly Tyr Val Leu Trp Leu Cys  
275 280 285  
Trp Cys Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Val  
10 290 295 300  
Gly Gly Met Cys Leu Ala Ile Ile Cys Phe Val Phe Ala Gln Lys  
15 305 310 315  
Leu Arg Leu Pro Gln Leu Gln Thr Gly Lys Ile Leu Arg Trp Glu  
320 325 330  
20 Tyr Glu Phe Val Ile His Gly His Gly Leu Ser Glu Lys Thr Ser  
335 340 345  
Asn Ser Leu Ala Arg Thr Gly Ser Pro Tyr Leu Leu Gly Arg Asp  
25 350 355 360  
Ser Phe Thr Gln Asn Pro Asn Ala Val Ala Phe Met Ser Gly Leu  
365 370 375  
30 Asn Asn Met Glu Leu Ala Asn Thr Asp His Glu Trp Ser Val Gly  
380 385 390  
Ser Ser Ser Pro Glu Pro Leu Pro Ser Pro Ala Ala Asp Leu Ile  
35 395 400 405  
Asp Arg Pro Ala Ser Thr Thr Ser Ser Ile Phe Asp Ala Ser His  
410 415 420  
40 Leu Pro

SEQ ID NO : 5

45 SEQUENCE LENGTH : 2340

SEQUENCE TYPE : nucleic acid

50 STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

5	TTCTTTCTG TCAAAGAATA ATAAAGTGCC CATCACTGTT CATATTGTT ACAAAAGTGGT	60
10	TTTCTGATTG GGTACTACTG CAGAGGCCGA TTTTTGCTT CAGTTACCAT AGCGTAAGAA	120
15	CACTAGCGAC TTTTGTTCGT GAACCAACAG AGTAGGATT CTACTGCTAC ATCTCTTAGG	180
20	TAGTTGGTTA GTCCGATCGC TCACTTTGG TTGTTGTTAA GTACTTCATA AGTTTATCCT	240
25	TTTCCTTTT CACACTGAGC TACTTTGGGT ATAGCTTTG GCCCAAGGAT CTTGAATT	300
30	TCTCCAAAAG TACTTTATTT TATATCCTAC AGGTTGCCGT TTTCATATTT TAAAAAGCTT	360
35	TTTAATCATT CCTTTGCCGA TGCGAAACCC TTTTCGAGA TGTTTCTAT CAGAGAGACC	420
40	TCCAAACTGC CATGTAGCCG ATTTAGAAAC AAGTTAGAT CCCCATCAA CGTTGTTGAA	480
45	GGTGCAAAAA TACAAACCCG CTTTAAGCGA CTGGGTGCAT TACATCTTCT TGGGATCCAT	540
50	CATGCTGTT GTGTTCATTA CTAATCCGC ACCTTGGATC TTCAAGATCC TTTTTATTG	600
55	TTTCTTGGGC ACTTTATTCA TCATTCAGC TACGTACAG TTTTCTTCA ATGCCCTGCC	660
60	CATCCTAACCA TGGGTGGCGC TGTATTTCAC TTCATCGTAC TTTCCAGATG ACCGCAGGCC	720
65	TCCTATTACT GTCAAAGTGT TACCAGCGGT GGAAACAATT TTATACGGCG ACAATTAAAG	780
70	TGATATTCTT GCAACATCGA CGAATTCCTT TTTGGACATT TTAGCATGGT TACCGTACGG	840
75	ACTATTCAT TATGGGGCCC CATTGTCGT TGCTGCCATC TTATTGTTAT TTGGTCCACC	900
80	AACTGTTTG CAAGGTTATG CTTTGCATT TGGTTATATG AACCTGTTG GTGTTATCAT	960
85	GCAAAATGTC TTTCCAGCCG CTCCCCCATG GTATAAAATT CTCTATGGAT TCCAATCAGC	1020
90	CAACTATGAT ATGCATGGCT CGCCTGGTGG ATTAGCTAGA ATTGATAAGC TACTCGGTAT	1080
95	TAATATGTAT ACTACAGCTT TTTCAAATTC CTCCGTCATT TTGGTGCCTT TTCTTCACT	1140
100	GCATTCCGGG TGTGCTACTA TGGAAGCCCT GTTTTCTGT TATTGTTTTC CAAAATTGAA	1200
105	GCCCTTGTGTT ATTGCTTATG TTTGCTGGTT ATGGTGGTCA ACTATGTATC TGACACACCA	1260
110	TTATTTGTA GACCTTATGG CAGGTTCTGT GCTGTCATAC GTTATTTCC AGTACACAAA	1320
115	GTACACACAT TTACCAATTG TAGATACATC TCTTTTTGC AGATGGTCAT ACACCTCAAT	1380
120	TGAGAAATAC GATATATCAA AGAGTGATCC ATTGGCTGCA GATTCAAACG ATATCGAAAG	1440
125	TGTCCTCTTG TCCAACTTGG AACTGACTT TGATCTTAAT ATGACTGATG AACCCAGTGT	1500
130	AAGCCCTTCG TTATTTGATG GATCTACTTC TGTTTCTCGT TCGTCCGCCA CGTCTATAAC	1560
135	GTCACTAGGT GTAAAGAGGG CTTAATGAGT ATTTTATCTG CAATTACCGA TACGGTTGGT	1620

5 CTTATGAGA TACATATAAA TATATATCTT TTTCTTCTT TTTCTTAGTC AGGATTGTG 1680  
 TTTAGCATAA TATACATGTA GTTTATTTAA TCACATACCA CTGATTATCT TTAGAATT 1740  
 ATAAATTTT GAAATAATG GGTGGCTTT AATGGGTCT ATGTTAAGTG AGGCTTTAG 1800  
 10 AATGCTCTTC CTGCTTGTT TATTATATGT GTATGAAAGA TATGTATGTA TTACATGTG 1860  
 TTGTTAGCGT CCCCCAGTCAA AACCTGTGCG CTATACCTAA ATGGATTGAT AATCTTCATT 1920  
 CACTAATTCT AAAATAGACT TCTTCCCCAA AGAACGGTGT AACGATGAGG CTCTATCCAG 1980  
 CTGCTTATCT AAATCAACTT TAACGATGGA TGATCTTATG ACACGGGGAT CTTTCTTTAA 2040  
 15 AGTTCTTAGA ATTTCAGACT GTACCGCAGC TGATGAATCA AACAGCATTAA AAAAGTGATA 2100  
 TGCTCGAAAA TGTTTTCTT GGTCTTCTT CATTATTTA GGAAGATAACC TTATGCCAT 2160  
 GGGTACAATG TCCCTCACCA CACCTCTGTT TTGAATAATC AGTTTCCGA TTGTGGAAGA 2220  
 20 CAATTCTTT GCTTCCAACCT TTGGCGCATT GGAGTTGGTT ATGCCAACAA GTCCGATCAG 2280  
 CTCATAAAGC ATCTTAGTGA AAAGGGTGGT TTTGCCTTAT TCTTCTCT GTTGAAGCTT 2340

25 SEQ ID NO : 6

SEQUENCE LENGTH : 401

SEQUENCE TYPE : amino acid

30 STRANDED : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

35 SEQUENCE DESCRIPTION :

Met Ala Asn Pro Phe Ser Arg Trp Phe Leu Ser Glu Arg Pro Pro

1 5 10 15

40 Asn Cys His Val Ala Asp Leu Glu Thr Ser Leu Asp Pro His Gln

20 25 30

45 Thr Leu Leu Lys Val Gln Lys Tyr Lys Pro Ala Leu Ser Asp Trp

35 40 45

50 Val His Tyr Ile Phe Leu Gly Ser Ile Met Leu Phe Val Phe Ile

55 60

55 Thr Asn Pro Ala Pro Trp Ile Phe Lys Ile Leu Phe Tyr Cys Phe

55

	65	70	75
5	Leu Gly Thr Leu Phe Ile Ile Pro Ala Thr Ser Gln Phe Phe Phe		
	80	85	90
	Asn Ala Leu Pro Ile Leu Thr Trp Val Ala Leu Tyr Phe Thr Ser		
10	95	100	105
	Ser Tyr Phe Pro Asp Asp Arg Arg Pro Pro Ile Thr Val Lys Val		
	110	115	120
15	Leu Pro Ala Val Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asp		
	125	130	135
	Ile Leu Ala Thr Ser Thr Asn Ser Phe Leu Asp Ile Leu Ala Trp		
20	140	145	150
	Leu Pro Tyr Gly Leu Phe His Tyr Gly Ala Pro Phe Val Val Ala		
	155	160	165
25	Ala Ile Leu Phe Val Phe Gly Pro Pro Thr Val Leu Gln Gly Tyr		
	170	175	180
	Ala Phe Ala Phe Gly Tyr Met Asn Leu Phe Gly Val Ile Met Gln		
30	185	190	195
	Asn Val Phe Pro Ala Ala Pro Pro Trp Tyr Lys Ile Leu Tyr Gly		
	200	205	210
35	Leu Gln Ser Ala Asn Tyr Asp Met His Gly Ser Pro Gly Gly Leu		
	215	220	225
	Ala Arg Ile Asp Lys Leu Leu Gly Ile Asn Met Tyr Thr Thr Ala		
40	230	235	240
	Phe Ser Asn Ser Ser Val Ile Phe Gly Ala Phe Pro Ser Leu His		
	245	250	255
45	Ser Gly Cys Ala Thr Met Glu Ala Leu Phe Phe Cys Tyr Cys Phe		
	260	265	270
	Pro Lys Leu Lys Pro Leu Phe Ile Ala Tyr Val Cys Trp Leu Trp		
50	275	280	285

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Trp Ser Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Met  
290 295 300  
5 Ala Gly Ser Val Leu Ser Tyr Val Ile Phe Gln Tyr Thr Lys Tyr  
305 310 315  
Thr His Leu Pro Ile Val Asp Thr Ser Leu Phe Cys Arg Trp Ser  
10 320 325 330  
Tyr Thr Ser Ile Glu Lys Tyr Asp Ile Ser Lys Ser Asp Pro Leu  
335 340 345  
15 Ala Ala Asp Ser Asn Asp Ile Glu Ser Val Pro Leu Ser Asn Leu  
350 355 360  
Glu Leu Asp Phe Asp Leu Asn Met Thr Asp Glu Pro Ser Val Ser  
20 365 370 375  
Pro Ser Leu Phe Asp Gly Ser Thr Ser Val Ser Arg Ser Ser Ala  
380 385 390  
25 Thr Ser Ile Thr Ser Leu Gly Val Lys Arg Ala  
395 400  
30

SEQ ID NO : 7

SEQUENCE LENGTH : 2340

35 SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

TOPOLOGY : linear

40 MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

45 TTTCTTTCTG TCAAAGAATA ATAAAGTGCC CATCAGTGTT CATATTTGTT ACAAAAGTGGT 60  
TTTCTGATTG GGTACTACTG CAGAGCCGTA TTTTTTGCTT CAGTTACCAT AGCGTAAGAA 120  
CACTAGCGAC TTTTGTTCGT GAACCAACAG AGTAGGATTCTACTGCTAC ATCTCTTAGG 180  
TAGTTGGTTA GTCCGATCGC TCACCTTTGG TTGTTGTTAA GTACTTCATA AGTTTATCCT 240  
50 TTTCCCTTTT CACACTGAGC TACTTTGGGT ATAGCTTTG GCCCAAGGAT CTTTGAATT 300

5 TCTCCAAAAG TACTTTATT TATATCCTAC AGGTTGCGGT TTTCATATTT TAAAAAGCTT 360  
 TTAATCATT CCTTTGCGTA TGGCAAACCC TTTTCGAGA TGGTTCTAT CAGAGAGACC 420  
 TCCAAACTGC CATGTAGCGG ATTTAGAAC AAGTTAGAT CCCCATCAA CGTTGTTGAA 480  
 GGTGCAAAAA TACAAACCCG CTTTAAGCGA CTGGGTGCAT TACATCTTCT TGGGATCCAT 540  
 CATGCTGTT GTGTTCATTA CTAATCCCGC ACCTTGGATC TTCAAGATCC TTTTTTATTG 600  
 TTTCTGGGC ACTTTATTCA TCATTCCAGC TACGTACAG TTTTCTTCA ATGCCTGCC 660  
 CATCCTAACCA TGGGTGGCGC TGTATTCAC TTCATCGTAC TTTCCAGATG ACCGCAGGCC 720  
 15 TCCTATTACT GTCAAAGTGT TACCAGCGGT GGAAACAAATT TTATACGGCG ACAATTAAAG 780  
 TGATATTCTT GCAACATCGA CGAACATCCTT TTTGGACATT TTAGCATGGT TACCGTACGG 840  
 ACTATTCAT TTTGGGGCCC CATTGTCGT TGCTGCCATC TTATTCGTAT TTGGTCCACC 900  
 20 AACTGTTTG CAAGGTTATG CTTTGCAATT TGTTTATATG AACCTGTTG GTGTTATCAT 960  
 GCAAAATGTC TTTCCAGCCG CTCCCCCATG GTATAAAATT CTCTATGGAT TGCAATCAGC 1020  
 CAACTATGAT ATGCATGGCT CGCCTGGTGG ATTAGCTAGA ATTGATAAGC TACTCGGTAT 1080  
 TAATATGTAT ACTACAGCTT TTTCAAATT CTCGTCATT TTCGGTGCTT TTCCCTCACT 1140  
 GCATTCCGGG TGTGCTACTA TGGAAGCCCT GTTTTCTGT TATTGTTTCA CAAAATTGAA 1200  
 GCCCTTGTAA ATTGCTTATG TTTGCTGGTT ATGGTGGTCA ACTATGTATC TGACACACCA 1260  
 30 TTATTTGTA GACCTTATGG CAGGTTCTGT GCTGTCATAC GTTATTTCC AGTACACAAA 1320  
 GTACACACAT TTACCAATTG TAGATACATC TCTTTTTGC AGATGCTCAT ACACCTCAAT 1380  
 TGAGAAATAC GATATATCAA AGAGTGATCC ATTGGCTGCA GATTCAAACG ATATCGAAAG 1440  
 35 TGTCCCTTG TCCAACCTGG AACTTGACTT TGATCTTAAT ATGACTGATG AACCCAGTGT 1500  
 AAGCCCTTCG TTATTTGATG GATCTACTTC TGTTTCTCGT TCGTCCGCCA CGTCTATAAC 1560  
 GTCACTAGGT GTAAAGAGGG CTTAATGAGT ATTTTATCTG CAATTACCGA TACGGTTGGT 1620  
 40 CTTATGTAGA TACATATAAA TATATATCTT TTTCTTTCTT TTTCTTAGTC AGGATTGTGG 1680  
 TTTAGCATAA TATACATGTA GTTTATTAA TCACATACCA CTGATTATCT TTACAATTAA 1740  
 ATAAATTTT GAAATAAAATG GGTGGCTTT AATGGTGTCT ATGTTAAGTG AGGCTTTAG 1800  
 45 AATGCTCTTC CTGCTTTGTT TATTATATGT GTATGAAAGA TATGTATGTA TTTACATGTG 1860  
 TTTGTAGCGT CCCCCAGTCAA AACCTGTGCG CTATACCTAA ATGGATTGAT AATCTTCATT 1920  
 CACTAATTCT AAAATAGACT TCTTCCCCAA AGAACGGTGT AACGATGAGG CTCTATCCAG 1980  
 50 CTGCTTATCT AAATCAACTT TAACGATGGA TGATCTTATG ACACGGGGAT CTTTCTTTAA 2040

AGTTCTTAGA ATTCAGACT GTACCCGAGC TGATGAATCA AACAGCATTAAAAAGTGATA 2100  
 5 TGCTCGAAAAA TGTTTTCTT GGTCTTCCTT CATTATTTA GGAAGATACC TTATGCCCAT 2160  
 GGGTACAATG TCCCTCACCA CACCTCTGTT TTGAATAATC AGTTTCCCGA TTGTGGAAGA 2220  
 CAATTCTTTT GCTTCCAACCT TTGGCGCATT GGAGTTGGTT ATGCCAACAA GTCCGATCAG 2280  
 CTCATAAAGC ATCTTAGTGA AAAGGGTGGT TTTGCGTTAT TCTTCCCTCT GTTGAAGCTT 2340

10

SEQ ID NO : 8

SEQUENCE LENGTH : 401

15

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

20

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ala Asn Pro Phe Ser Arg Trp Phe Leu Ser Glu Arg Pro Pro

25

1 5 10 15

Asn Cys His Val Ala Asp Leu Glu Thr Ser Leu Asp Pro His Gln

30

20 25 30

Thr Leu Leu Lys Val Gln Lys Tyr Lys Pro Ala Leu Ser Asp Trp

35 40 45

35

Val His Tyr Ile Phe Leu Gly Ser Ile Met Leu Phe Val Phe Ile

50 55 60

40

Thr Asn Pro Ala Pro Trp Ile Phe Lys Ile Leu Phe Tyr Cys Phe

65 70 75

45

Leu Gly Thr Leu Phe Ile Ile Pro Ala Thr Ser Gln Phe Phe Phe

80 85 90

50

Asn Ala Leu Pro Ile Leu Thr Trp Val Ala Leu Tyr Phe Thr Ser

95 100 105

Ser Tyr Phe Pro Asp Asp Arg Arg Pro Pro Ile Thr Val Lys Val

110 115 120

55

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	Leu Pro Ala Val Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asp		
5	125	130	135
	Ile Leu Ala Thr Ser Thr Asn Ser Phe Leu Asp Ile Leu Ala Trp		
	140	145	150
10	Leu Pro Tyr Gly Leu Phe His Phe Gly Ala Pro Phe Val Val Ala		
	155	160	165
	Ala Ile Leu Phe Val Phe Gly Pro Pro Thr Val Leu Gln Gly Tyr		
15	170	175	180
	Ala Phe Ala Phe Gly Tyr Met Asn Leu Phe Gly Val Ile Met Gln		
	185	190	195
20	Asn Val Phe Pro Ala Ala Pro Pro Trp Tyr Lys Ile Leu Tyr Gly		
	200	205	210
	Leu Gln Ser Ala Asn Tyr Asp Met His Gly Ser Pro Gly Gly Leu		
25	215	220	225
	Ala Arg Ile Asp Lys Leu Leu Gly Ile Asn Met Tyr Thr Thr Ala		
	230	235	240
30	Phe Ser Asn Ser Ser Val Ile Phe Gly Ala Phe Pro Ser Leu His		
	245	250	255
	Ser Gly Cys Ala Thr Met Glu Ala Leu Phe Phe Cys Tyr Cys Phe		
35	260	265	270
	Pro Lys Leu Lys Pro Leu Phe Ile Ala Tyr Val Cys Trp Leu Trp		
	275	280	285
40	Trp Ser Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Met		
	290	295	300
	Ala Gly Ser Val Leu Ser Tyr Val Ile Phe Gln Tyr Thr Lys Tyr		
45	305	310	315
	Thr His Leu Pro Ile Val Asp Thr Ser Leu Phe Cys Arg Trp Ser		
	320	325	330
50	Tyr Thr Ser Ile Glu Lys Tyr Asp Ile Ser Lys Ser Asp Pro Leu		

	335	340	345
5	Ala Ala Asp Ser Asn Asp Ile Glu Ser Val Pro Leu Ser Asn Leu		
	350	355	360
	Glu Leu Asp Phe Asp Leu Asn Met Thr Asp Glu Pro Ser Val Ser		
10	365	370	375
	Pro Ser Leu Phe Asp Gly Ser Thr Ser Val Ser Arg Ser Ser Ala		
	380	385	390
15	Thr Ser Ile Thr Ser Leu Gly Val Lys Arg Ala		
	395	400	

20 SEQ ID NO : 9

SEQUENCE LENGTH : 5340

SEQUENCE TYPE : nucleic acid

25 STRANDEDNESS : double

TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

30 SEQUENCE DESCRIPTION :

AGCGCTTCTA	TTTTCCCTCCC	CACCGCGAGG	CGGAAATGGC	ACATTTTTT	TCTTTTGCTT	60	
CTGTGCTTT	GCTGTAATT	TTGGCATGTG	CTATTGTATG	AAGATAACGC	GTGGTTCCGT	120	
35	GGAAATAGCC	GGAAATTGG	CCGGGAATAT	GACGGACATG	ATTTAACACC	CGTGGAAATG	180
	AAAAAAGCCA	AGGTAAGAAA	GTGGCAATAT	TTTCCTACA	AATAGATCTG	CTGTCCCTTA	240
	GATGATTACC	ATACATATAT	ATATTTATTA	CACACATCTG	TCAGAGGTAG	CTAGCGAAGG	300
40	TGTCACTGAA	ATATTTTTG	TTCCAGTTAG	TATAAATACG	GAGGTAGAAC	AGCTCTCCGC	360
	GTGTATATCT	TTTTTGCGC	TATACAAGAA	CAGGAAGAAC	GCATTCCAT	ACCTTTTCT	420
45	CCTTACAGGT	GCCCTCTGAG	TAGTGTACG	AACGAGGAAA	AAGATTAATA	TTACTGTTT	480
	TATATTCAAA	AAGAGTAAAG	CCGTTGCTAT	ATACGAATAT	GACGATTACC	GTGGGGGATG	540
	CAGTTTCGGA	GACGGAGCTG	GAAAACAAAA	GTCAAAACGT	GGTACTATCT	CCCAAGGCAT	600
50	CTGCTTCTTC	AGACATAAGC	ACAGATGTTG	ATAAAGACAC	ATCGTCTTCT	TGGGATGACA	660
	AATCTTGCT	GCCTACAGGT	GAATATATTG	TGGACAGAAA	TAAGCCCCAA	ACCTACTTGA	720

ATAGCGATGA TATCGAAAAA GTGACAGAAT CTGATATTT CCCTCAGAAA CGTCTGTTT 780  
 CATTCTGCA CTCTAAGAAA ATTCCAGAAG TACCACAAAC CGATGACGAG AGGAAGATAT 840  
 5 ATCCTCTGTT CCATACAAAT ATTATCTCTA ACATGTTTT TTGGTGGGTT CTACCCATCC 900  
 TGCAGTTGG TTATAAGAGA ACGATACAGC CGAACGATCT CTTCAAAATG GATCCGAGGA 960  
 TGTCTATAGA GACCCTTAT GACGACTTG AAAAAAACAT GATTTACTAT TTTGAGAAGA 1020  
 10 CGAGGAAAAA ATACCGTAAA AGACATCCAG AAGCGACAGA AGAAGAGGTT ATGGAAAATG 1080  
 CCAAAC TAAACATACA GTTCTGAGAG CTTTATTATT CACTTTAAG AAACAGTACT 1140  
 TCATGTCGAT AGTGTGTTGCA ATTCTCGCTA ATTGTACATC CGGTTTAAC CCCATGATTA 1200  
 15 CCAAGAGGCT AATTGAGTTT GTCGAAGAAA AGGCTATTT TCATAGCATG CATGTTAAC 1260  
 AAGGTATTGG TTACGCTATT CGTGCATGTT TGATGATGTT CGTTAACGGG TTGACGTTCA 1320  
 ATCATTCTT TCATACATCC CAACTGACTG GTGTGCAAGC TAAGTCTATT CTTACTAAAG 1380  
 20 CTGCCATGAA GAAAATGTT AATGCATCTA ATTATGCGAG ACATTGTTT CCTAACGGTA 1440  
 AAGTGA CTTTCAGCTTAC ACAGATCTCG CTAGAATTCA ATTTGCCCTA TCTTTTCAGC 1500  
 CGTTTTGGC TGGGTTCCCT GCAATTGGG CTATTTGCAT TGTTTATTG ATCGTTAAC 1560  
 25 TTGGACCCAT TGCCTTAGTT GGGATTGGTA TTTTTTCGG TGGGTTTTC ATATCCTTAT 1620  
 TTGCATTAA GTTAATTCTG GGCTTAGAA TTGCTGCGAA CATCTTCACT GATGCTAGAG 1680  
 30 TTACCATGAT GAGAGAACTG CTGAATAATA TAAAAATGAT TAAATATTAT ACGTGGGAGG 1740  
 ATGCGTATGA AAAAAATATT CAAGATATTA GGACCAAAGA GATTCTAAA GTAGAAAAA 1800  
 TGCAACTATC AAGAAATTTC TTGATTGCTA TGCCCATGTC TTTGCCTAGT ATTGCTTCAT 1860  
 35 TGGTCAC TTTGCCTTT CTTGCAATG TACAAAGTTA ATAAAGGAGG CAGGCAACCT GGTAATATTT 1920  
 TTGCCTCTT ATCTTTATT CAGGTCTTGA GTTGCCTAAAT GTTTTCTTA CCTATTGCTA 1980  
 TTGGTACTGG AATTGACATG ATCATTGGAT TGGGCCGTT GCAAAGCTTA TTGGAGGCTC 2040  
 40 CAGAAGATGA TCCAAATCAG ATGATTGAAA TGAAGCCCTC TCCTGGCTT GATCCAAAT 2100  
 TGGCTCTAA AATGACACAT TGCTCATTG AGTGGGAAGA TTATGAATTA AACGACGCTA 2160  
 TTGAAGAAGC AAAAGGAGAA GCTAAAGATG AAGGTAAAAA GAACAAAAAA AAGCGTAAGG 2220  
 45 ATACATGGGG TAAGCCATCT GCAAGTACTA ATAAGGCAGA AAGATTGGAC AATATGTTGA 2280  
 AAGACAGAGA CGGCCCCGAA GATTTAGAAA AAACCTTCGTT TAGGGTTTC AAGGACTTGA 2340  
 ACTTCGATAT TAAAAAGGGC GAATTATTA TGATTACGGG ACCTATTGGT ACTCGTAAAT 2400  
 50 CTTCATTATT GAATGCGATG GCAGGATCAA TGAGAAAAAT TGATGGTAAG GTGAAAGTCA 2460

5 ACGGGGACTT ATTAATGTGT GGTTATCCAT GGATTCAAAA TGCATCTGTA AGAGATAACA 2520  
 TCATATTCGG TTCACCATTC AATAAAGAAA AGTATGATGA AGTAGTTCGT GTTGCTCTT 2580  
 TGAAAGCTGA TCTGGATATT TTACCGGCAG CGCATATGAC CGAAATTGGG GAACGTGGTA 2640  
 TTACTTTATC TGGTGGTCAA AAGGCACGTA TCAATTAGC CAGGTCTGTT TATAAGAAGA 2700  
 AGGATATTAA TGTATTGAC GATGTCCTAA CTGCTGTCGA TTCTCGTGT GGAAACACAA 2760  
 10 TCATGGATGA ATGTCTAACCG GGAATGCTTG CTAATAAAAC CAGAATTAA GCAACGCATC 2820  
 AGTTGTCACT GATTGAGAGA GCTTCTAGAG TCATCGTTT AGGTACTGAT GCCAAGTCG 2880  
 15 ATATTGGTAC TGTTGATGAG CTAAAGCTC GTAATCAAAC TTTGATAAAT CTTTACAAT 2940  
 TCTCTTCTCA AAATTCCGGAG AAAGAGGATG AAGAACACGGA AGCGGTTGTT TCCGGTGAAT 3000  
 TGGGACAAC TAAATATGAA CCAGAGGTAA AGGAATTGAC TGAACGTAAAG AAAAAGGCTA 3060  
 20 CAGAAATGTC ACAAACTGCA AATACTGGTA AAATTGTCAGC GGATGGTCAT ACTAGTAGTA 3120  
 AAGAAGAAAG AGCAGTCAAT AGTATCAGTC TGAAATATA CCGTGAATAC ATAAAGCTG 3180  
 CAGTAGGTAA GTGGGGTTT ATCCCACTAC CGTTGTATGC AATTTAGTC GTTGGAACCA 3240  
 25 CATTCTGCTC ACTTTTTCT TCCGTTGGT TATCTTACTG GACTGAGAAT AAATTCAAAA 3300  
 ACAGACCACC CAGTTTTAT ATGGGTCTTT ACTCCTTCTT TGTGTTGCT GCTTTCATAT 3360  
 TCATGAATGG CCAGTTCAAC ATACTTGCG CAATGGGTAT TATGGCATCG AAATGGTTAA 3420  
 30 ATTTGAGGGC TGTGAAAAGA ATTTACACA CTCCAATGTC ATACATAGAT ACCACACCTT 3480  
 TGGGACGTAT TCTGAACAGA TTCACAAAAG ATACAGATAAG CTTAGATAAT GAGTTAACCG 3540  
 AAAGTTTACG GTTGATGACA TCTCAATTG CTAATATTGT AGGTGTTGC GTCATGTGTA 3600  
 35 TTGTTTACTT GCGGTGGTTT GCTATCGCAA TTCCGTTCTT TTTGGTCATC TTTGTTCTGA 3660  
 TTGCTGATCA TTATCAGAGT TCTGGTAGAG AAATTAAAAG ACTTGAAGCT GTGCAACGGT 3720  
 CTTTGTGTTA CAATAATTAA AATGAAGTTT TGGGTGGGAT GGATACAATC AAAGCATACC 3780  
 40 GAAGTCAGGA ACCATTTTG GCGAAATCAG ATTTTTGAT CAACAAGATG AATGAGGCAG 3840  
 GATACTTGT AGTTGTCCTG CAAACATGGG TAGGTATTT CCTTGATATG GTTGCTATCG 3900  
 CATTGCACT AATTATTACG TTATTGTGT TTACGAGAGC CTTTCCATT TCCGGGGCTT 3960  
 45 CAGTTGGTGT TTTGTTGACT TATGTATTAC AATTGCCTGG TCTATTAAAT ACCATTTAA 4020  
 GGGCAATGAC TCAAACAGAG AATGACATGA ATAGTGCCGA AAGATTGGTA ACATATGCAA 4080  
 CTGAACCTACC ACTAGAGGCA TCCTATAGAA AGCCCGAAAT GACACCTCCA GACTCATGGC 4140  
 50 CCTCAATGGG CGAAATAATT TTTGAAAATG TTGATTTGC CTATAGACCT GGTTTACCTA 4200

5 TAGTTTTAAA AAATCTAAC TTGAATATCA AGAGTGGGA AAAAATTGGT ATCTGTGGTC 4260  
 GTACAGGTGC TGGTAAGTCC ACTATTATGA GTGCCCTTA CAGGTTGAAT GAATTGACCG 4320  
 CAGGTAAAAT TTTAATTGAC AATGTTGATA TAAGTCAGCT GGGACTTTTC GATTAAGAA 4380  
 GAAAATTAGC CATCATTCCA CAAGATCCAG TATTATTTAG GGGTACGATT CGCAAGAACT 4440  
 10 TAGATCCATT TAATGAGCGT ACAGATGACG AATTATGGGA TGCATTGGTG AGAGGTGGTG 4500  
 CTATGCCAA GGATGACTTG CCGGAAGTGA ATTGCAAAA ACCTGATGAA AATGGTACTC 4560  
 ATGGTAAAAT GCATAAGTTC CATTAGATC AAGCAGTGGA AGAAGAGGGC TCCAATTCT 4620  
 15 CCTTAGGTGA GAGACAACTA TTAGCATTAA CAAGGGCATT GGTCCGCCAA TCAAAAATAT 4680  
 TGATTTGGA TGAGGCTACA TCCTCAGTGG ACTACGAAAC GGATGGCAAA ATCCAAACAC 4740  
 GTATTGTTGA CGAATTGGA CATTGTACAA TTTTGTGTAT TGCTCACAGA CTGAAGACCA 4800  
 20 TTGTAAATTA TGATCGTATT CTTGTTTAG AGAAGGGTGA AGTCGCAGAA TTCGATACAC 4860  
 CATGGACGTT GTTGTCAA GAAGATAGTA TTTTCAGAAG CATGTGTCT AGATCTGGTA 4920  
 TTGTGGAAAA TGATTCGAG AACAGAAGTT AATTATATT ATTTGTTGCA TGATTTTCT 4980  
 25 CTTTATTTA TTTATATGTT GCCGATGGTA CAAATTAGTA CTAGAAAAGA AAACCCACTA 5040  
 CTATGACTTG CAGAAAAACT TATGTGTGCC ATAGATAGAT ATAATTGCAT ACCCACATCG 5100  
 TATACTCAAA ATTCCGAAAA GAACATTCA TTTTTATGA GGCAAACTGA ACAACGCTTC 5160  
 30 GGTCCCTTTT TCATTCTAGA AATATATATT TATACATCAT TTTCAGAAGA TATTCAAAGA 5220  
 ACTTATTGGG ATGTCTATT ACTGAATAAA GTATACACAA AAAACGAATT TAAAATGGAA 5280  
 GGCATAAATA GAAAACCTAG AAGTGAAAAT CCTAAAACCG AAGGATATT CAAATACGTA 5340  
 35

SEQ ID NO : 10

SEQUENCE LENGTH : 1477

SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Thr Ile Thr Val Gly Asp Ala Val Ser Glu Thr Glu Leu Glu

50 5

10 10

15 15

	Asn Lys Ser Gln Asn Val Val Leu Ser Pro Lys Ala Ser Ala Ser		
5	20	25	30
	Ser Asp Ile Ser Thr Asp Val Asp Lys Asp Thr Ser Ser Ser Trp		
	35	40	45
10	Asp Asp Lys Ser Leu Leu Pro Thr Gly Glu Tyr Ile Val Asp Arg		
	50	55	60
15	Asn Lys Pro Gln Thr Tyr Leu Asn Ser Asp Asp Ile Glu Lys Val		
	65	70	75
	Thr Glu Ser Asp Ile Phe Pro Gln Lys Arg Leu Phe Ser Phe Leu		
	80	85	90
20	His Ser Lys Lys Ile Pro Glu Val Pro Gln Thr Asp Asp Glu Arg		
	95	100	105
	Lys Ile Tyr Pro Leu Phe His Thr Asn Ile Ile Ser Asn Met Phe		
25	110	115	120
	Phe Trp Trp Val Leu Pro Ile Leu Arg Val Gly Tyr Lys Arg Thr		
	125	130	135
30	Ile Gln Pro Asn Asp Leu Phe Lys Met Asp Pro Arg Met Ser Ile		
	140	145	150
	Glu Thr Leu Tyr Asp Asp Phe Glu Lys Asn Met Ile Tyr Tyr Phe		
35	155	160	165
	Glu Lys Thr Arg Lys Lys Tyr Arg Lys Arg His Pro Glu Ala Thr		
	170	175	180
40	Glu Glu Glu Val Met Glu Asn Ala Lys Leu Pro Lys His Thr Val		
	185	190	195
	Leu Arg Ala Leu Leu Phe Thr Phe Lys Lys Gln Tyr Phe Met Ser		
45	200	205	210
	Ile Val Phe Ala Ile Leu Ala Asn Cys Thr Ser Gly Phe Asn Pro		
	215	220	225
50	Met Ile Thr Lys Arg Leu Ile Glu Phe Val Glu Glu Lys Ala Ile		

	230	235	240
5	Phe His Ser Met His Val Asn Lys Gly Ile Gly Tyr Ala Ile Gly		
	245	250	255
	Ala Cys Leu Met Met Phe Val Asn Gly Leu Thr Phe Asn His Phe		
10	260	265	270
	Phe His Thr Ser Gln Leu Thr Gly Val Gln Ala Lys Ser Ile Leu		
	275	280	285
15	Thr Lys Ala Ala Met Lys Lys Met Phe Asn Ala Ser Asn Tyr Ala		
	290	295	300
20	Arg His Cys Phe Pro Asn Gly Lys Val Thr Ser Phe Val Thr Thr		
	305	310	315
	Asp Leu Ala Arg Ile Glu Phe Ala Leu Ser Phe Gln Pro Phe Leu		
	320	325	330
25	Ala Gly Phe Pro Ala Ile Leu Ala Ile Cys Ile Val Leu Leu Ile		
	335	340	345
	Val Asn Leu Gly Pro Ile Ala Leu Val Gly Ile Gly Ile Phe Phe		
30	350	355	360
	Gly Gly Phe Phe Ile Ser Leu Phe Ala Phe Lys Leu Ile Leu Gly		
	365	370	375
35	Phe Arg Ile Ala Ala Asn Ile Phe Thr Asp Ala Arg Val Thr Met		
	380	385	390
	Met Arg Glu Val Leu Asn Asn Ile Lys Met Ile Lys Tyr Tyr Thr		
40	395	400	405
	Trp Glu Asp Ala Tyr Glu Lys Asn Ile Gln Asp Ile Arg Thr Lys		
	410	415	420
45	Glu Ile Ser Lys Val Arg Lys Met Gln Leu Ser Arg Asn Phe Leu		
	425	430	435
50	Ile Ala Met Ala Met Ser Leu Pro Ser Ile Ala Ser Leu Val Thr		
	440	445	450

	Phe Leu Ala Met Tyr Lys Val Asn Lys Gly Gly Arg Gln Pro Gly		
	455	460	465
5	Asn Ile Phe Ala Ser Leu Ser Leu Phe Gln Val Leu Ser Leu Gln		
	470	475	480
	Met Phe Phe Leu Pro Ile Ala Ile Gly Thr Gly Ile Asp Met Ile		
10	485	490	495
	Ile Gly Leu Gly Arg Leu Gln Ser Leu Leu Glu Ala Pro Glu Asp		
15	500	505	510
	Asp Pro Asn Gln Met Ile Glu Met Lys Pro Ser Pro Gly Phe Asp		
	515	520	525
20	Pro Lys Leu Ala Leu Lys Met Thr His Cys Ser Phe Glu Trp Glu		
	530	535	540
	Asp Tyr Glu Leu Asn Asp Ala Ile Glu Glu Ala Lys Gly Glu Ala		
25	545	550	555
	Lys Asp Glu Gly Lys Lys Asn Lys Lys Lys Arg Lys Asp Thr Trp		
	560	565	570
30	Gly Lys Pro Ser Ala Ser Thr Asn Lys Ala Lys Arg Leu Asp Asn		
	575	580	585
	Met Leu Lys Asp Arg Asp Gly Pro Glu Asp Leu Glu Lys Thr Ser		
35	590	595	600
	Phe Arg Gly Phe Lys Asp Leu Asn Phe Asp Ile Lys Lys Gly Glu		
	605	610	615
40	Phe Ile Met Ile Thr Gly Pro Ile Gly Thr Gly Lys Ser Ser Leu		
	620	625	630
	Leu Asn Ala Met Ala Gly Ser Met Arg Lys Ile Asp Gly Lys Val		
45	635	640	645
	Glu Val Asn Gly Asp Leu Leu Met Cys Gly Tyr Pro Trp Ile Gln		
50	650	655	660
	Asn Ala Ser Val Arg Asp Asn Ile Ile Phe Gly Ser Pro Phe Asn		

	665	670	675
5	Lys Glu Lys Tyr Asp Glu Val Val Arg Val Cys Ser Leu Lys Ala		
	680	685	690
	Asp Leu Asp Ile Leu Pro Ala Gly Asp Met Thr Glu Ile Gly Glu		
10	695	700	705
	Arg Gly Ile Thr Leu Ser Gly Gly Gln Lys Ala Arg Ile Asn Leu		
	710	715	720
15	Ala Arg Ser Val Tyr Lys Lys Asp Ile Tyr Val Phe Asp Asp		
	725	730	735
	Val Leu Ser Ala Val Asp Ser Arg Val Gly Lys His Ile Met Asp		
20	740	745	750
	Glu Cys Leu Thr Gly Met Leu Ala Asn Lys Thr Arg Ile Leu Ala		
	755	760	765
25	Thr His Gln Leu Ser Leu Ile Glu Arg Ala Ser Arg Val Ile Val		
	770	775	780
	Leu Gly Thr Asp Gly Gln Val Asp Ile Gly Thr Val Asp Glu Leu		
30	785	790	795
	Lys Ala Arg Asn Gln Thr Leu Ile Asn Leu Leu Gln Phe Ser Ser		
	800	805	810
35	Gln Asn Ser Glu Lys Glu Asp Glu Glu Gln Glu Ala Val Val Ser		
	815	820	825
	Gly Glu Leu Gly Gln Leu Lys Tyr Glu Pro Glu Val Lys Glu Leu		
40	830	835	840
	Thr Glu Leu Lys Lys Ala Thr Glu Met Ser Gln Thr Ala Asn		
	845	850	855
45	Ser Gly Lys Ile Val Ala Asp Gly His Thr Ser Ser Lys Glu Glu		
	860	865	870
	Arg Ala Val Asn Ser Ile Ser Leu Lys Ile Tyr Arg Glu Tyr Ile		
50	875	880	885

Lys Ala Ala Val Gly Lys Trp Gly Phe Ile Ala Leu Pro Leu Tyr  
 890 895 900  
 5 Ala Ile Leu Val Val Gly Thr Thr Phe Cys Ser Leu Phe Ser Ser  
 905 910 915  
 10 Val Trp Leu Ser Tyr Trp Thr Glu Asn Lys Phe Lys Asn Arg Pro  
 920 925 930  
 15 Pro Ser Phe Tyr Met Gly Leu Tyr Ser Phe Phe Val Phe Ala Ala  
 935 940 945  
 20 Phe Ile Phe Met Asn Gly Gln Phe Thr Ile Leu Cys Ala Met Gly  
 950 955 960  
 25 Ile Met Ala Ser Lys Trp Leu Asn Leu Arg Ala Val Lys Arg Ile  
 965 970 975  
 30 Leu His Thr Pro Met Ser Tyr Ile Asp Thr Thr Pro Leu Gly Arg  
 980 985 990  
 35 Ile Leu Asn Arg Phe Thr Lys Asp Thr Asp Ser Leu Asp Asn Glu  
 995 1000 1005  
 40 Leu Thr Glu Ser Leu Arg Leu Met Thr Ser Gln Phe Ala Asn Ile  
 1010 1015 1020  
 45 Val Gly Val Cys Val Met Cys Ile Val Tyr Leu Pro Trp Phe Ala  
 1025 1030 1035  
 50 Ile Ala Ile Pro Phe Leu Leu Val Ile Phe Val Leu Ile Ala Asp  
 1040 1045 1050  
 His Tyr Gln Ser Ser Gly Arg Glu Ile Lys Arg Leu Glu Ala Val  
 1055 1060 1065  
 Gln Arg Ser Phe Val Tyr Asn Asn Leu Asn Glu Val Leu Gly Gly  
 1070 1075 1080  
 Met Asp Thr Ile Lys Ala Tyr Arg Ser Gln Glu Arg Phe Leu Ala  
 1085 1090 1095  
 55 Lys Ser Asp Phe Leu Ile Asn Lys Met Asn Glu Ala Gly Tyr Leu

	1100	1105	1110
5	Val Val Val Leu Gln Arg Trp Val Gly Ile Phe Leu Asp Met Val		
	1115	1120	1125
	Ala Ile Ala Phe Ala Leu Ile Ile Thr Leu Leu Cys Val Thr Arg		
10	1130	1135	1140
	Ala Phe Pro Ile Ser Ala Ala Ser Val Gly Val Leu Leu Thr Tyr		
	1145	1150	1155
15	Val Leu Gln Leu Pro Gly Leu Leu Asn Thr Ile Leu Arg Ala Met		
	1160	1165	1170
	Thr Gln Thr Glu Asn Asp Met Asn Ser Ala Glu Arg Leu Val Thr		
20	1175	1180	1185
	Tyr Ala Thr Glu Leu Pro Leu Glu Ala Ser Tyr Arg Lys Pro Glu		
	1190	1195	1200
25	Met Thr Pro Pro Glu Ser Trp Pro Ser Met Gly Glu Ile Ile Phe		
	1205	1210	1215
	Glu Asn Val Asp Phe Ala Tyr Arg Pro Gly Leu Pro Ile Val Leu		
30	1220	1225	1230
	Lys Asn Leu Asn Leu Asn Ile Lys Ser Gly Glu Lys Ile Gly Ile		
	1235	1240	1245
35	Cys Gly Arg Thr Gly Ala Gly Lys Ser Thr Ile Met Ser Ala Leu		
	1250	1255	1260
	Tyr Arg Leu Asn Glu Leu Thr Ala Gly Lys Ile Leu Ile Asp Asn		
40	1265	1270	1275
	Val Asp Ile Ser Gln Leu Gly Leu Phe Asp Leu Arg Arg Lys Leu		
	1280	1285	1290
45	Ala Ile Ile Pro Gln Asp Pro Val Leu Phe Arg Gly Thr Ile Arg		
	1295	1300	1305
	Lys Asn Leu Asp Pro Phe Asn Glu Arg Thr Asp Asp Glu Leu Trp		
50	1310	1315	1320

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Asp Ala Leu Val Arg Gly Gly Ala Ile Ala Lys Asp Asp Leu Pro  
1325 1330 1335  
5 Glu Val Lys Leu Gln Lys Pro Asp Glu Asn Gly Thr His Gly Lys  
1340 1345 1350  
Met His Lys Phe His Leu Asp Gln Ala Val Glu Glu Gly Ser  
10 1355 1360 1365  
Asn Phe Ser Leu Gly Glu Arg Gln Leu Leu Ala Leu Thr Arg Ala  
1370 1375 1380  
15 Leu Val Arg Gln Ser Lys Ile Leu Ile Leu Asp Glu Ala Thr Ser  
1385 1390 1395  
20 Ser Val Asp Tyr Glu Thr Asp Gly Lys Ile Gln Thr Arg Ile Val  
1400 1405 1410  
Glu Glu Phe Gly Asp Cys Thr Ile Leu Cys Ile Ala His Arg Leu  
25 1415 1420 1425  
Lys Thr Ile Val Asn Tyr Asp Arg Ile Leu Val Leu Glu Lys Gly  
1430 1435 1440  
30 Glu Val Ala Glu Phe Asp Thr Pro Trp Thr Leu Phe Ser Gln Glu  
1445 1450 1455  
Asp Ser Ile Phe Arg Ser Met Cys Ser Arg Ser Gly Ile Val Glu  
35 1460 1465 1470  
Asn Asp Phe Glu Asn Arg Ser  
1475  
40 SEQ ID NO : 11  
SEQUENCE LENGTH : 26  
SEQUENCE TYPE : nucleic acid  
45 STRANDEDNESS : single  
TOPOLOGY : linear  
MOLECULE TYPE : Other nucleic acid(synthetic DNA)  
50 SEQUENCE DESCRIPTION :

TTTGGTTAYA TGAAYYTNTT YGGNGT 26

5 SEQ ID NO : 12  
SEQUENCE LENGTH : 29  
SEQUENCE TYPE : nucleic acid  
10 STRANDEDNESS : single  
TOPOLOGY : linear  
15 MOLECULE TYPE : Other nucleic acid(synthetic DNA)  
SEQUENCE DESCRIPTION :  
TCTACAAART ARTGGTGNCT NARRTACAT 29

20 SEQ ID NO : 13  
SEQUENCE LENGTH : 2274  
SEQUENCE TYPE : nucleic acid  
25 STRANDEDNESS : double  
TOPOLOGY : linear  
30 MOLECULE TYPE : genomic DNA  
SEQUENCE DESCRIPTION :  
TTATATATAT TATTGATTG TTCCCTGTTGT TATTTAGTTT AGAATCAGAC GACTACACCA 60  
35 GAACCACAAT TCAACCAACA CTTATATAGA ACCTGGCTTG GAAAAAAAGTA ACATTTATCA 120  
TTCCTATACT TTTTTAGCAA ACATAATCCG TGTTTTACAT ATATTATTCA CCCAATATCA 180  
TAACAAAAAC AAACTGAATA ATGGCGTCTT CTATTTGCG TTCCAAAATA ATACAAAAAC 240  
40 CGTACCAATT ATTCCACTAC TATTTCTTC TGGAGAAGGC TCCTGGTTCT ACAGTTAGTG 300  
ATTGAAATT TGATACAAAC ATACAAACGA GTTTACGTAA ATAAAGCAT CATCATTGGA 360  
45 CGGTGGGAGA AATATTCCAT TATGGGTTTT TGGTTTCCAT ACTTTTTTC GTGTTGTGG 420  
TTTTCCCAGC TTCATTTTT ATAAAATTAC CAATAATCTT AGCATTGCT ACTTGTTTTT 480  
TAATACCCCTT AACATCACAA TTTTTCTTC CTGCCTTGCC CGTTTCACT TGGTTGGCAT 540  
50 TATATTTAC GTGTGCTAAA ATACCTCAAG AATGGAAACC AGCTATCACA GTTAAAGTTT 600  
TACCAAGCTAT GGAAACAATT TTGTACGGCG ATAATTATC AAATGTTTG GCAACCATCA 660

55

5 CTACCGGAGT GTTAGATATA TTGGCATGGT TACCATATGG GATTATTCA TTCAGTTCC 720  
CATTGTACT TGCTGCTATT ATATTTTAT TTGGGCCACC GACGGCATTAGATCATTG 780  
GATTGCCCT TGGTTATATG AACTTGCTTG GAGTCTTGAT TCAAATGGCA TTCCCAGCTG 840  
CTCCTCCATG GTACAAAAAC TTGCACGGAT TAGAACCAGC TAATTATTCA ATGCACGGGT 900  
10 CTCCCTGGTGG ACTTGGAACG ATAGATAAAT TGTTAGGTGT TGATATGTAT ACCACAGGGT 960  
TTTCCAATTC ATCAATCATT TTTGGGCAT TCCCATCGTT ACATTCAAGA TGTTGTATCA 1020  
TGGAACTGTT ATTTTGTTGT TGTTGTTTC CACGATTCAA GTTTGTGTGG GTTACATACG 1080  
15 CATCTTGGCT TTGGTGGAGC ACGATGTATT TGACCCATCA CTACTTGTC GATTGATTG 1140  
GTGGAGCCAT GCTATCTTG ACTGTTTTG AGTCACCAA ATATAAATAT TTGCCAAAAAA 1200  
ACAAAGAACGG CCTTTCTGT CGTTGGTCAT ACACTGAAAT TGAAAAAAATC GATATCCAAG 1260  
20 AGATTGACCC TTTATCATAAC AATTATATCC CTGTCAACAG CAATGATAAT GAAAGCAGAT 1320  
TGTATACGAG AGTGTACCAA GAGTCTCAGG TTAGTCCCCC ACAGAGAGCT GAAACACCTG 1380  
AAGCATTGAA GATGTCAAAT TTTCTAGGT CTAGACAAAG CTCAAAGACT CAGGTTCCAT 1440  
25 TGAGTAATCT TACTAACAAAT GATCAAGTGT CTGGAATTAA CGAAGAGGAT GAAGAAGAAC 1500  
AAGGGCATGA AATTTCATCG AGTACTCCTT CGGTGTTGA AGACCAACCA CAGGGTAGCA 1560  
CATATGCTGC ATCCTCAGCT ACATCACTAG ATGATTTGGA TTCCAAAAGA ATTAGTAAA 1620  
30 ATAACAGTTT CTATTAATTCTT CTTTATTCC TCCTAATTAA TGATTTATG CTCAATACCT 1680  
ACACTATCTG TTTTTAATTCTT CCTACTTTT TTTTATTATT GTTGAGTTCA TTTGCTGTTC 1740  
ATTGAATATT TACAATTTCG CTTAACATTAC CATCAATATA GAATGGGCAC AGTTTTTTA 1800  
35 AGTTTTTG TTTTTGTGTT TGTCTTCTT TTTTACATT AATGTGTTG GATTGTTTA 1860  
GGTTCCCTTA TCCCTTAGCC CCCTCAGAAT ACTATTTAT CTAATTAATT TGTGTTTATT 1920  
TTCTGATATT TACCAATTGC TTTTCTTTT GGATATTTAT AATAGCATCC CCTAATAATT 1980  
40 AATATACAAC TGTTTCATAT ATATACGTGT ATGTCCTGTA GTGGTGGAAA CTGGACTCAA 2040  
CATTGTATT AATGTGTACA AGAAAGCAGT GTTAATGCTA CTATTATAAT TTTGAGGTG 2100  
CAAATCAAGA GGTTGGCAGC TTTCTTATGG CTATGACCGT GAATGAAGGC TTGAAACCA 2160  
45 CGTAATAAAC AAAAGCCAAAC AAGTTTTTT AGAGCCTTTA ACAACATACG CAATGAGAGT 2220  
GATTGCAATA CTACAAGATA TAGCCCCAAA AATTGAATGC ATTTCAACAA CAAC 2274

SEQ ID NO : 14

SEQUENCE LENGTH : 471

SEQUENCE TYPE : amino acid

5 STRANDEDNESS : single

TOPOLOGY : linear

10 MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met Ala Ser Ser Ile Leu Arg Ser Lys Ile Ile Gln Lys Pro Tyr

15 5 10 15

Gln Leu Phe His Tyr Tyr Phe Leu Ser Gln Lys Ala Pro Gly Ser

20 25 30

Thr Val Ser Asp Leu Asn Phe Asp Thr Asn Ile Gln Thr Ser Leu

25 35 40 45

Arg Lys Leu Lys His His His Trp Thr Val Gly Glu Ile Phe His

50 55 60

Tyr Gly Phe Leu Val Ser Ile Leu Phe Phe Val Phe Val Val Phe

65 70 75

Pro Ala Ser Phe Phe Ile Lys Leu Pro Ile Ile Leu Ala Phe Ala

30 80 85 90

Thr Cys Phe Leu Ile Pro Leu Thr Ser Gln Phe Phe Leu Pro Ala

55 95 100 105

Leu Pro Val Phe Thr Trp Leu Ala Leu Tyr Phe Thr Cys Ala Lys

110 115 120

Ile Pro Gln Glu Trp Lys Pro Ala Ile Thr Val Lys Val Leu Pro

40 125 130 135

Ala Met Glu Thr Ile Leu Tyr Gly Asp Asn Leu Ser Asn Val Leu

45 140 145 150

Ala Thr Ile Thr Thr Gly Val Leu Asp Ile Leu Ala Trp Leu Pro

155 160 165

50 Tyr Gly Ile Ile His Phe Ser Phe Pro Phe Val Leu Ala Ala Ile

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	170	175	180
5	Ile Phe Leu Phe Gly Pro Pro Thr Ala Leu Arg Ser Phe Gly Phe		
	185	190	195
	Ala Phe Gly Tyr Met Asn Leu Leu Gly Val Leu Ile Gin Met Ala		
10	200	205	210
	Phe Pro Ala Ala Pro Pro Trp Tyr Lys Asn Leu His Gly Leu Glu		
	215	220	225
15	Pro Ala Asn Tyr Ser Met His Gly Ser Pro Gly Gly Leu Gly Arg		
	230	235	240
	Ile Asp Lys Leu Leu Gly Val Asp Met Tyr Thr Thr Gly Phe Ser		
20	245	250	255
	Asn Ser Ser Ile Ile Phe Gly Ala Phe Pro Ser Leu His Ser Gly		
	260	265	270
25	Cys Cys Ile Met Glu Val Leu Phe Leu Cys Trp Leu Phe Pro Arg		
	275	280	285
	Phe Lys Phe Val Trp Val Thr Tyr Ala Ser Trp Leu Trp Trp Ser		
30	290	295	300
	Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Ile Gly Gly		
	305	310	315
35	Ala Met Leu Ser Leu Thr Val Phe Glu Phe Thr Lys Tyr Lys Tyr		
	320	325	330
	Leu Pro Lys Asn Lys Glu Gly Leu Phe Cys Arg Trp Ser Tyr Thr		
40	335	340	345
	Glu Ile Glu Lys Ile Asp Ile Gin Glu Ile Asp Pro Leu Ser Tyr		
	350	355	360
45	Asn Tyr Ile Pro Val Asn Ser Asn Asp Asn Glu Ser Arg Leu Tyr		
	365	370	375
	Thr Arg Val Tyr Gin Glu Ser Gin Val Ser Pro Pro Gin Arg Ala		
50	380	385	390

Glu Thr Pro Glu Ala Phe Glu Met Ser Asn Phe Ser Arg Ser Arg  
 395 400 405  
 5 Gln Ser Ser Lys Thr Gln Val Pro Leu Ser Asn Leu Thr Asn Asn  
 410 415 420  
 10 Asp Gln Val Ser Gly Ile Asn Glu Glu Asp Glu Glu Glu Gly  
 425 430 435  
 15 Asp Glu Ile Ser Ser Thr Pro Ser Val Phe Glu Asp Glu Pro  
 440 445 450  
 20 Gln Gly Ser Thr Tyr Ala Ala Ser Ser Ala Thr Ser Val Asp Asp  
 455 460 465  
 25 Leu Asp Ser Lys Arg Asn  
 470

SEQ ID NO : 15

SEQUENCE LENGTH : 243

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

30 TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

SEQUENCE DESCRIPTION :

35 TTTGAAAAAT TTGAATTTA AAATTAATCC AATGGAAAAA ATTGGTATTT GTGGAAGAAC 60  
 CGGTGCTGGT AAATCATCAA TTATGACAGC ATTATATCGA TTATCAGAAT TAGAACTGGG 120  
 GAAAATTATT ATTGATGATA TTGATATTTC AACTTTGGGT TTAAAAGATC TTGATCAAA 180  
 40 ATTATCAATT ATTCCTCAAG ATCCAGTATT ATTCCGAGGT TCAATTGGA AAAACTTGGA 240  
 TCC 243

45 SEQ ID NO : 16

SEQUENCE LENGTH : 80

50 SEQUENCE TYPE : amino acid

STRANDEDNESS : single

5 TOPOLOGY : linear

5 MOLECULE TYPE : peptide

5 SEQUENCE DESCRIPTION :

Leu Lys Asn Leu Asn Phe Lys Ile Asn Pro Met Glu Lys Ile Gly		
5	10	15
10 Ile Cys Gly Arg Thr Gly Ala Gly Lys Ser Ser Ile Met Thr Ala		
20	25	30
15 Leu Tyr Arg Leu Ser Glu Leu Glu Leu Gly Lys Ile Ile Ile Asp		
35	40	45
20 Asp Ile Asp Ile Ser Thr Leu Gly Leu Lys Asp Leu Arg Ser Lys		
50	55	60
25 Leu Ser Ile Ile Pro Gln Asp Pro Val Leu Phe Arg Gly Ser Ile		
65	70	75
30 Arg Lys Asn Leu Asp		
80		

5 SEQ ID NO : 17

30 SEQUENCE LENGTH : 1601

SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

35 TOPOLOGY : linear

MOLECULE TYPE : genomic DNA

ANTI-SENSE : Yes

40 SEQUENCE DESCRIPTION :

AGGAAGATGA CTTGCATCAA AGATGGAGGA AGTGGTACTG GCAGGACGAT CAATCAAATC	60
AGCAGCAGGA CTAGGTAACG GCTCAGGTGA TGATGAACCC ACGGACCATT CATGATCGGT	120
45 GTTAGCAAGT TCCATATTGT TAAGACCACT CATGAAGGCT ACTGCATTAG GGTTTTGAGT	180
AAAAGAATCC CTTCCAAGTA AGTATGGCT GCCGGTACCA GCCAAGGAGT TGCTGGTTT	240
TTCGGAAAGA CCATGACCGT GGATAACAAA CTCGTATTCC CAACGAAGGA TTTTACCACT	300
50 TTGCAACTGT GGGAGGCCGT A GCTTTGAGC AAAAACGAAG CATATAATAG C TAAACACAT	360

5	ACCGCCGACC AAATCTACAA AGTAGTGGTG GGTAAGGTAC ATAGTACACC AGCAAAGCCA	420
10	TAGAACATAT CCATAAAAGC AGAAGCGGTA TCGAGGAAAC ACATGCGAAA GGAAAAGTGC	480
15	TTCCAGCATG GCCCATCCAG CGTGAAGAGA TGGAAAGGCA CCAAAAACAA CCGGAGAGTT	540
20	AGAAAAAACCA TCAGTGTAAA TGCTAGTGCC GAAGAGAGCA TCAATACGGG CCAATCCACC	600
25	AGGAGAGCCA CGTACTGCAT ACGTGGCAGG TTCTAAACCA TACATATTTCATAACCAAGG	660
30	AGGAGAACAG GGGAAAGCCA TTTGGATAAG AACACCAAAT AAATTCAAT AACCAAAAGT	720
35	TCGAGCCCCAA ACTGGAAGAG TTCCAGGAGG TGCAGGATG AAAAGAATAA ATGAAATGAT	780
40	AAAAGGAGCC GAATAATGCA TGACTCCATA TGGAAACCCAG GCCAAAATAT CAAGGATGCT	840
45	ATGCGTGGTT TTCGAGAGAA GACTAGAAAG ATTAGAGCCA TAAAGAATAT TTTCAAGTGT	900
50	GGGTAAAACA CGAACCCATA TGGGTGGACG CCAGCGTTCT GGAATAAACCTACAAGAGTA	960
55	AAATAAAATT GCCCAGGTGA TGATAACAAT GGCAGGAAAA AAAATTGGC GTGTTAAAGG	1020
60	AACGGTCAAC GCAATGGCCA AAAGACAGGC AATGCCAAAT TTCCCCCAGA ATCCAGGAGA	1080
65	TTCAATGACA ATACAAGCAA AAATCAAATT ACCTGCTAGA AACACATATT GCAAATGTGT	1140
70	CCATGACCAT TTCGTATTGC GTAGCAAACG AAATGTAGGC ATAGGGTTA AGCTTGTTC	1200
75	CAACTTGTAT TGGGATGCTC GGTTACACGC AGCAAGGCAG TTTTTAAGG TCGAAAGAGC	1260
80	AGACATTGCT TCAAAGAATT ATCAGAGTAA AAAAGGGAAG CCTACGAAAA AAATTTCGTA	1320
85	AGGAATTAAC CGGAAAAACTA AAGGAAAAAA AAGGAATTTC TATGAAGGAA AGAAAGTAGC	1380
90	TATTAAATGC AAGTGTCAAG CACTAAAAG TAGCGATGTA AAATATTAA AAAAAGATGG	1440
95	ACCGATTAAC CAATGTTCAAG CTCACAGTTG CCAGCAATCA GGGCTATTT TTTTTTTT	1500
100	TTATAAAATT GCTAATTATA TATAATATAA TTAGTTATT AACTTGCTTT TCCTCAAAAA	1560
105	ACCAATTGCA GAAAGGAAC TTTGCAGAGG CAAAAAAGCT T	1601
110	SEQ ID NO : 18	
115	SEQUENCE LENGTH : 1601	
120	SEQUENCE TYPE : nucleic acid	
125	STRANDEDNESS : double	
130	TOPOLOGY : linear	
135	MOLECULE TYPE : mRNA	
140	ANTI-SENSE : Yes	

## SEQUENCE DESCRIPTION :

5	AGGAAGAUGA CUUGCAUCA AGAUGGAGGA AGUGGUACUG GCAGGACGAU CAAUCAAAUC	60
	AGCAGCAGGA CUAGGUACG GCUCAGGUGA UGAUGAACCC ACGGACCAUU CAUGAUCGGU	120
	GUUAGCAAGU UCCAUAUUGU UAAGACCACU CAUGAAGGCC ACUGCAUUAG GGUUUUGAGU	180
10	AAAAGAAUCC CUUCCAAGUA AGUAUGGGCU CCCGGUACGA GCCAAGGAGU UGCUGGUUUU	240
	UUCGGAAAGA CCAUGACCGU GGAAUACAAA CUCGUAUUCC CAACGAAGGA UUUUACCAGU	300
	UUGCAACUGU GGGAGGCGUA GCUUUUGAGC AAAAACGAAG CAUAAUAAUG CUAAACACAU	360
15	ACCGCCGACC AAAUCUACAA AGUAGUGGUG GGUAAGGUAC AUAGUACACC AGCAAAGCCA	420
	UAGAACAUAU CCAUAAAAGC AGAACCGGUU UCGAGGAAAC ACAUGCGAAA GGAAAAGUGC	480
	UUCCAGCAUG GCCCAUCCAG CGUGAAGAGA UGGAAAGGCC CCAAAACAA CCGGAGAGUU	540
20	AGAAAAACCA UCAGUGUAAA UGCUAGUGCC GAAGAGAGCA UCAAUACGGG CCAAUCCACC	600
	AGGAGAGCCA CGUACUGCAU ACGUGGCAGG UUCUAAACCA UACAUUUUU CAUACCAAGG	660
	AGGAGAACAG GGGAAAGCCA UUUGGUAAG AACACCAAAU AAAUCAUAU AACCAAAAGU	720
25	UCGAGCCAA ACUGGAAGAG UUCCAGGAGG UGCAAAGAUG AAAAGAAUAA AUGAAAUGAU	780
	AAAAGGAGCC GAAUAAUGCA UGACUCCAUA UGGAACCCAG GCCAAAAUAU CAAGGAUGCU	840
	AUGCGUGGUU UUCGAGAGAA GACUAGAAAG AUUAGAGCCA UAAAGAAUAU UUCAAGUGU	900
30	GGGUAAAACA CGAACCCAAU UGGGUGGACG CCAGCGUUCU GGAAUAAACC UACAAGAGUA	960
	AAAUAAAAUU GCCCAGGUGA UGAUAACAAU GGCAGGAAAA AAAAUUUGGC GUGUUAAGG	1020
	AACGGUCAAC GCAAUGGCCA AAAGACAGGC AAUGCCAAU UUCCCCAGA AUCCAGGAGA	1080
35	UUCAAUGACA AUACAAGCAA AAAUCAAAUU ACCUGCUAGA AACACAUUU GCAAUUGUGU	1140
	CCAUGACCAU UUCGUAUUGC GUAGCAAACG AAAUGUAGGC AUAGGGUUUA AGCUUGUUUC	1200
	CAACUUGUAU UGGGAUGCUC GGUACACGC ACCAAGGCC UUUUUUAGG UCGAAAGAGC	1260
40	AGACAUUGCU UCAAAGAAUU AUCAGAGUA AAAAGGGAAG CGUACGAAAA AAAUUUCGUA	1320
	AGGAUUUAAC CGGAAAACUA AAGGAAAAAA AAGGAAUUUU UAUGAAGGAA AGAAAGUAGC	1380
	UAUUAAAUGC AAGUGUCAAG CACUAAAAG UAGCGAUGUA AAAUUUUAA AAAAGAUGG	1440
45	ACCGAUUAAC CAAUGUUCAG CUCACAGUUG CCAGCAAUC A GGGCUAUUU UUUUUUUUU	1500
	UUAUAAAAUU GCUAAUUAUA UAUAAUUAUA UUAGUUUAUU AACUUGCUUU UCCUAAAAA	1560
50	ACCAAUUCGA GAAAGGAACU UUUGCAGAGG CAAAAAAGCU U	1601

SEQ ID NO : 19

SEQUENCE LENGTH : 12

5 SEQUENCE TYPE : amino acid

STRANDEDNESS : single

TOPOLOGY : linear

10 MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Cys Phe Thr Ser Ser Tyr Phe Pro Asp Asp Arg Arg

15 5 10

SEQ ID NO : 20

SEQUENCE LENGTH : 19

20 SEQUENCE TYPE : amino acid

STRANDEDNESS : single

25 TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

30 Cys Tyr Thr Ser Ile Glu Lys Tyr Asp Ile Ser Lys Ser Asp Pro

5 10 15

Leu Ala Ala Asp

35

SEQ ID NO : 21

SEQUENCE LENGTH : 1553

40 SEQUENCE TYPE : nucleic acid

STRANDEDNESS : double

45 TOPOLOGY : linear

MOLECULE TYPE : Genomic DNA

SEQUENCE DESCRIPTION :

50 TTTTACATAT ATTATTCACC CAATATCATA ACAAAAACAA ACTGAATGAT GGCATCTTCT 60

ATTTTGCCTT CCAAAATAAT ACAAAAACCG TACCAATTAT TCCACTACTA TTTTCTTCTG 120

55

5 GAGAAGGCTC CTGGTTCTAC AGTTAGTGAT TTGAATTTG ATACAAACAT ACAAACGAGT 180  
 TTACGTAAAT TAAAGCATCA TCATTGGACG GTGGGAGAAA TATTCCATTA TGGGTTTTG 240  
 GTTCCATAC TTTTTTCGT GTTGTGGTT TTCCCAGCTT CATTTTTAT AAAATTACCA 300  
 ATAATCTTAG CATTGCTAC TTGTTTTA ATACCCCTAA CATCACAATT TTTCTTCCT 360  
 GCCTTGCCTG TTTTCACTG CTTGGCATTAA TATTTTACGT GTGCTAAAAT ACCTCAAGAA 420  
 TGGAAACCAG CTATCACAGT TAAAGTTTA CCAGCTATGG AAACAATTT GTACGGCGAT 480  
 AATTATCAA ATGTTTGCG AACCATCACT ACCGGAGTGT TAGATATATT GGCATGGTTA 540  
 10 CCATATGGGA TTATTCAATT CAGTTCCCA TTTGTACTTG CTGCTATTAT ATTTTTATTT 600  
 GGGCCACCGA CGGCATTAAG ATCATTGGA TTTGCCTTG GTTATATGAA CTTGCTTGGA 660  
 GTCTTGATTCA AAATGGCATT CCCAGCTGCT CCTCCATGGT ACAAAAACCTT GCACGGATT 720  
 15 GAACCAGCTA ATTATTCAAT GCACGGGTCT CCTGGTGGAC TTGGAAGGAT AGATAAATTG 780  
 TTAGGTGTTG ATATGTATAC CACAGGGTTT TCCAATTCAAT CAATCATTGTTT TGGGCCATT 840  
 CCATCGTTAC ATTCAAGGATG TTGTATCATG GAAGTGTATTTT TTTGTGTTG GTTGTGTTCCA 900  
 20 CGATTCAAGT TTGTGTGGGT TACATACGCA TCTTGGCTTT GGTGGAGCAC GATGTATTG 960  
 ACCCATCACT ACTTTGTCGA TTTGATTGGT GGAGCCATGC TATCTTGAC TGTGTTTGAA 1020  
 TTCACCAAAT ATAAATATTT GCCAAAAAAC AAAGAAGGCC TTTCTGTCG TTGGTCATAC 1080  
 25 ACTGAAATTG AAAAATCGA TATCCAAGAG ATTGACCCCTT TATCATAACAA TTATATCCCT 1140  
 GTCAACAGCA ATGATAATGA AAGCAGATTG TATACGAGAG TGTACCAAGA GCCTCAGGTT 1200  
 AGTCCCCCAC AGAGAGCTGA AACACCTGAA GCATTTGAGA TGTCAAATTT TTCTAGGTCT 1260  
 30 AGACAAAGCT CAAAGACTCA GGTTCCATTG ACTAATCTTA CTAACAATGA TCAAGTGCCT 1320  
 GGAATTAACG AAGAGGATGA AGAAGAAGAA GGCGATGAAA TTTCTGTCGAG TACTCCTTCG 1380  
 GTGTTGAAAG ACGAACCCACA GGGTAGCACA TATGCTGCAT CCTCAGCTAC ATCAGTAGAT 1440  
 35 GATTTGGATT CCAAAAGAAA TTAGTAAAC ACCAGTTCTT ATTAATTTCT TTATTCCTC 1500  
 CTAATTAATG ATTTTATGTT CAATACCTAC ACTATCTGTT TTTAATTTCC TAC 1553  
 40  
 45 SEQ ID NO : 22  
 SEQUENCE LENGTH : 472  
 50 SEQUENCE TYPE : amino acid  
 STRANDEDNESS : single

5  
TOPOLOGY : linear

MOLECULE TYPE : peptide

SEQUENCE DESCRIPTION :

Met	Met	Ala	Ser	Ser	Ile	Leu	Arg	Ser	Lys	Ile	Ile	Gln	Lys	Pro
1					5				10				15	
Tyr	Gln	Leu	Phe	His	Tyr	Tyr	Phe	Leu	Leu	Glu	Lys	Ala	Pro	Gly
					20				25				30	
Ser	Thr	Val	Ser	Asp	Leu	Asn	Phe	Asp	Thr	Asn	Ile	Gln	Thr	Ser
					35				40				45	
Leu	Arg	Lys	Leu	Lys	His	His	His	Trp	Thr	Val	Gly	Glu	Ile	Phe
					50				55				60	
His	Tyr	Gly	Phe	Leu	Val	Ser	Ile	Leu	Phe	Phe	Val	Phe	Val	Val
					65				70				75	
Phe	Pro	Ala	Ser	Phe	Phe	Ile	Lys	Leu	Pro	Ile	Ile	Leu	Ala	Phe
					80				85				90	
Ala	Thr	Cys	Phe	Leu	Ile	Pro	Leu	Thr	Ser	Gln	Phe	Phe	Leu	Pro
					95				100				105	
Ala	Leu	Pro	Val	Phe	Thr	Trp	Leu	Ala	Leu	Tyr	Phe	Thr	Cys	Ala
					110				115				120	
Lys	Ile	Pro	Gln	Glu	Trp	Lys	Pro	Ala	Ile	Thr	Val	Lys	Val	Leu
					125				130				135	
Pro	Ala	Met	Glu	Thr	Ile	Leu	Tyr	Gly	Asp	Asn	Leu	Ser	Asn	Val
					140				145				150	
Leu	Ala	Thr	Ile	Thr	Thr	Gly	Val	Leu	Asp	Ile	Leu	Ala	Trp	Leu
					155				160				165	
Pro	Tyr	Gly	Ile	Ile	His	Phe	Ser	Phe	Pro	Phe	Val	Leu	Ala	Ala
					170				175				180	
Ile	Ile	Phe	Leu	Phe	Gly	Pro	Pro	Thr	Ala	Leu	Arg	Ser	Phe	Gly
					185				190				195	

Phe Ala Phe Gly Tyr Met Asn Leu Leu Gly Val Leu Ile Gln Met  
 200 205 210  
 5 Ala Phe Pro Ala Ala Pro Pro Trp Tyr Lys Asn Leu His Gly Leu  
 215 220 225  
 Glu Pro Ala Asn Tyr Ser Met His Gly Ser Pro Gly Gly Leu Gly  
 10 230 235 240  
 Arg Ile Asp Lys Leu Leu Gly Val Asp Met Tyr Thr Thr Gly Phe  
 245 250 255  
 15 Ser Asn Ser Ser Ile Ile Phe Gly Ala Phe Pro Ser Leu His Ser  
 260 265 270  
 Gly Cys Cys Ile Met Glu Val Leu Phe Leu Cys Trp Leu Phe Pro  
 20 275 280 285  
 Arg Phe Lys Phe Val Trp Val Thr Tyr Ala Ser Trp Leu Trp Trp  
 290 295 300  
 25 Ser Thr Met Tyr Leu Thr His His Tyr Phe Val Asp Leu Ile Gly  
 305 310 315  
 Gly Ala Met Leu Ser Leu Thr Val Phe Glu Phe Thr Lys Tyr Lys  
 320 325 330  
 Tyr Leu Pro Lys Asn Lys Glu Gly Leu Phe Cys Arg Trp Ser Tyr  
 335 340 345  
 35 Thr Glu Ile Glu Lys Ile Asp Ile Gln Glu Ile Asp Pro Leu Ser  
 350 355 360  
 Tyr Asn Tyr Ile Pro Val Asn Ser Asn Asp Asn Glu Ser Arg Leu  
 365 370 375  
 40 Tyr Thr Arg Val Tyr Gln Glu Pro Gln Val Ser Pro Pro Gln Arg  
 380 385 390  
 45 Ala Glu Thr Pro Glu Ala Phe Glu Met Ser Asn Phe Ser Arg Ser  
 395 400 405  
 50 Arg Gln Ser Ser Lys Thr Gln Val Pro Leu Ser Asn Leu Thr Asn

	410	415	420
5	Asn Asp Gln Val Pro Gly Ile Asn Glu Glu Asp Glu Glu Glu		
	425	430	435
10	Gly Asp Glu Ile Ser Ser Ser Thr Pro Ser Val Phe Glu Asp Glu		
	440	445	450
15	Pro Gln Gly Ser Thr Tyr Ala Ala Ser Ser Ala Thr Ser Val Asp		
	455	460	465
	Asp Leu Asp Ser Lys Arg Asn		
	470		

## Claims

25 1. An isolated gene coding for a protein which regulates aureobasidin sensitivity.

30 2. An isolated gene as claimed in Claim 1 which is contained in a DNA fragment represented by a restriction enzyme map as specified in any of Fig. 1 to Fig. 3.

35 3. An isolated gene as claimed in Claim 1 which is hybridizable with a gene of Claim 2.

40 4. A process for cloning a gene of Claim 1 characterized by using a gene of Claim 2 or 3 or a part thereof as a probe.

45 5. A nucleic acid probe which comprises a sequence consisting of 15 or more bases and is hybridizable with a gene of Claim 1.

50 6. An antisense DNA of a gene which codes for a protein regulating aureobasidin sensitivity.

55 7. An antisense RNA of a gene which codes for a protein regulating aureobasidin sensitivity.

60 8. A recombinant plasmid containing a gene of Claim 1.

65 9. A transformant having a recombinant plasmid of Claim 8 introduced thereinto.

70 10. A process for producing a protein regulating aureobasidin sensitivity characterized by culturing a transformant of Claim 9 and collecting the protein regulating aureobasidin sensitivity from the culture.

75 11. An isolated protein regulating aureobasidin sensitivity which is encoded by a gene of Claim 1.

80 12. An antibody against a protein of Claim 11.

85 13. A process for detecting a protein regulating aureobasidin sensitivity which comprises using an antibody of Claim 12.

90 14. A process for detecting a gene coding for a protein regulating aureobasidin sensitivity which comprises the hybridization with the use of a nucleic acid probe of Claim 5.

**15. A process for screening an antimycotic which comprises using a transformant of Claim 9 or a protein of Claim 11.**

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Fig. 1

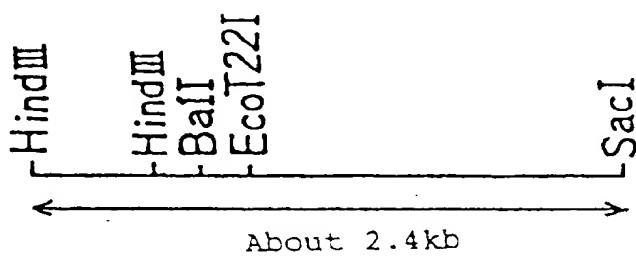


Fig. 2

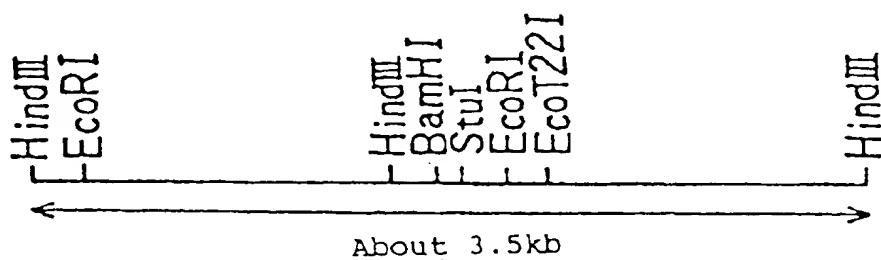


Fig. 3

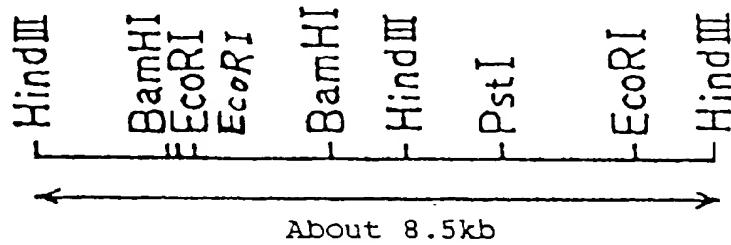


Fig. 4

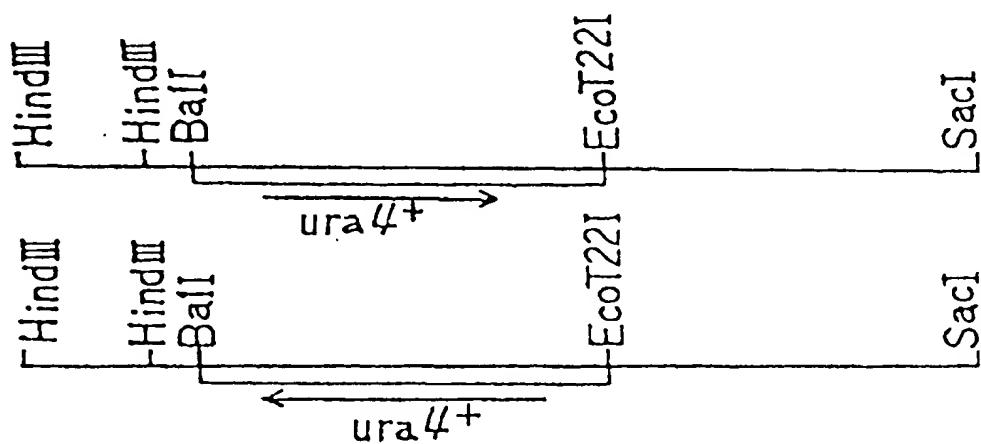


Fig. 5

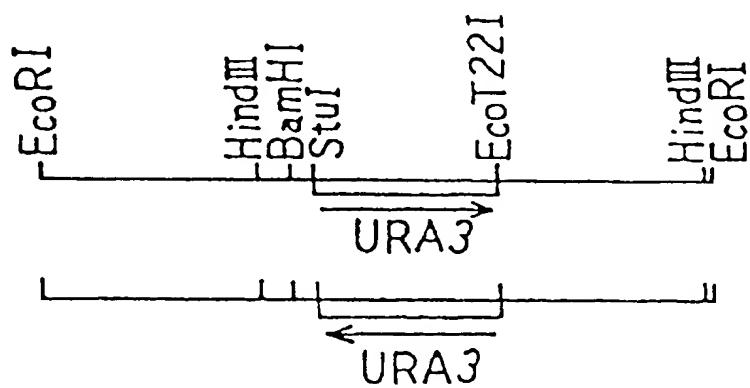


Fig. 6

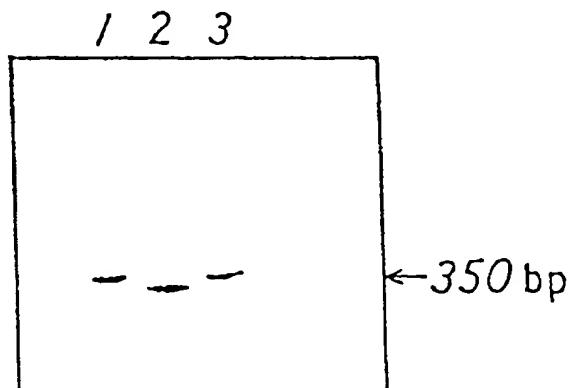


Fig. 7

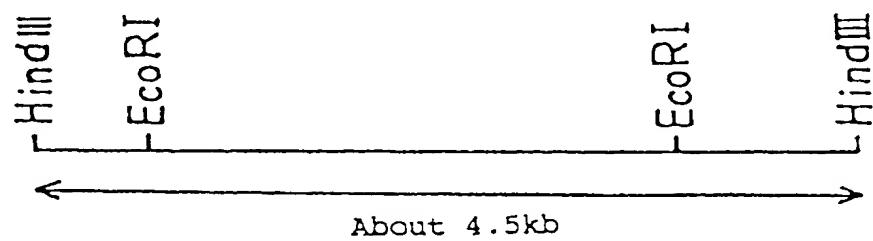


Fig. 8

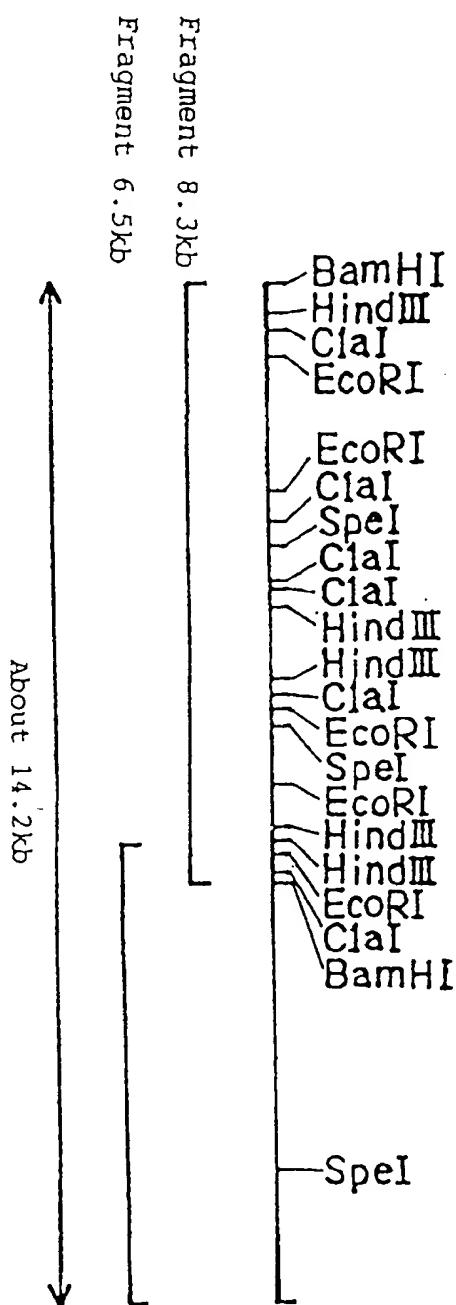


Fig. 9

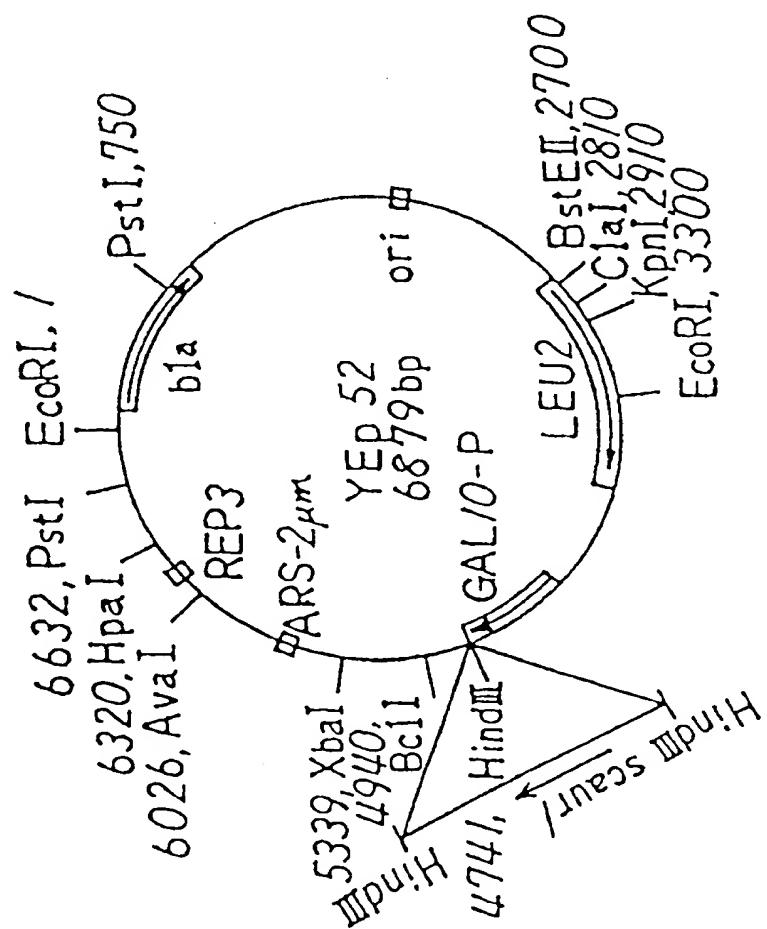


Fig. 10

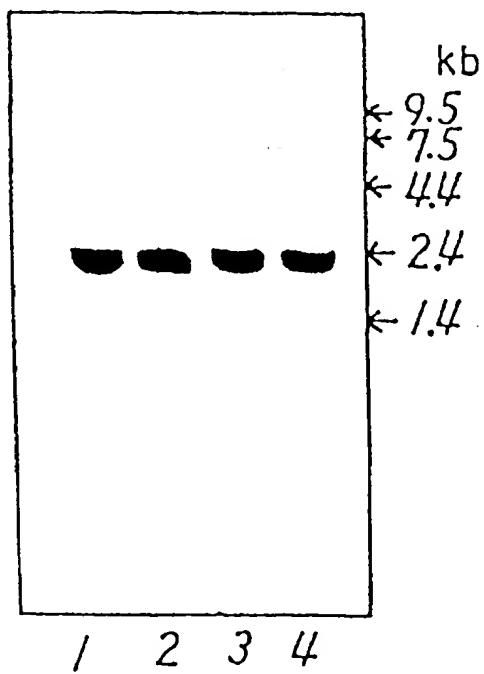


Fig. 11

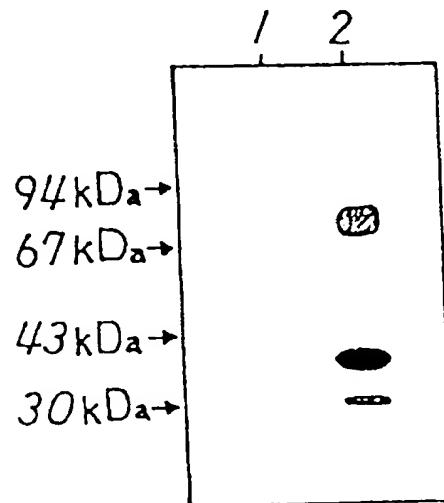
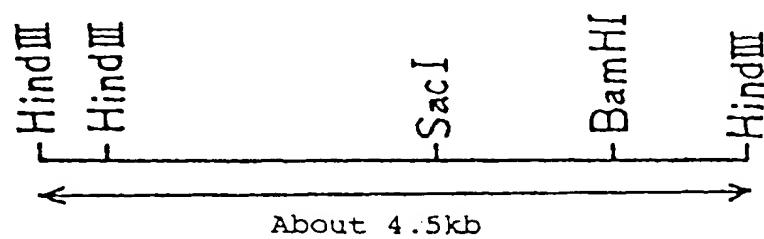


Fig. 12



(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets

(11)



EP 0 644 262 A3

(12)

## EUROPEAN PATENT APPLICATION

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• **Kazutoh, Takesako**  
Otsu-shi, Shiga-ken (JP)  
• **Ikunoshin, Kato**  
Uji-Shi, Kyoto-fu (JP)

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(74) Representative: **Wakerley, Helen Rachael**  
**Reddie & Grose,**  
**16 Theobalds Road**  
**London WC1X 8PL (GB)**

(71) Applicant: **TAKARA SHUZO CO. LTD.**  
**Fushimi-ku, Kyoto-shi, Kyoto-fu (JP)**

(72) Inventors:

- **Takashi, Okado**  
Otsu-shi, Shiga-ken (JP)

### (54) **Gene coding for a protein regulating aureobasidin sensitivity**

(57) An isolated gene coding for a protein regulating aureobasidin sensitivity. A process for cloning the gene with the use of the gene or a part of the same as a probe. A nucleic acid probe being hybridizable with the gene. An antisense DNA or RNA of the gene. A recombinant or transformant having the gene contained therein. An isolated protein regulating aureobasidin sensitivity and a process for producing the same by using the transformant. An antibody for the protein. A process for detecting the protein or the gene. A process for screening an antimycotic by using the protein or the transformant. This invention is useful in the diagnosis and treatment of diseases including mycoses.

EP 0 644 262 A3



## EUROPEAN SEARCH REPORT

Application Number

EP 94 30 3401

## DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)		
A	EP-A-0 443 719 (TAKARA SHUZO CO. LTD) ---		C12N15/31 C07K13/00 C12Q1/68 G01N33/569 C12N15/11		
A	MOL. GEN. GENET., vol. 235, 1992, pages 64-73, XP002007445 P. HAFFTER ET AL.: "Suppression of carboxy-terminal truncations of the yeast mitochondrial mRNA-specific translational activator PET122 by mutations in two new genes MRP17 and PET127" * figure 3 * -----	1			
TECHNICAL FIELDS SEARCHED (Int.Cl.5)					
C07K C12N C12Q G01N					
The present search report has been drawn up for all claims					
Place of search	Date of completion of the search	Examiner			
THE HAGUE	4 July 1996	Skelly, J			
CATEGORY OF CITED DOCUMENTS					
X : particularly relevant if taken alone	I : theory or principle underlying the invention				
Y : particularly relevant if combined with another document of the same category	E : earlier patent document, but published on, or after the filing date				
A : technological background	D : document cited in the application				
O : non-written disclosure	L : document cited for other reasons				
P : intermediate document	& : member of the same patent family, corresponding document				